

EXPERIENTIA



REVUE MENSUELLE DES SCIENCES PURES ET APPLIQUÉES
MONATSSCHRIFT FÜR DAS GESAMTE GEBIET DER NATURWISSENSCHAFT
RIVISTA MENSILE DI SCIENZE PURE E APPLICATE
MONTHLY JOURNAL OF PURE AND APPLIED SCIENCE

Editores:

P. HUBER · R. MATTHEY · A. v. MURALT · L. RUZICKA
Basel Lausanne Bern Zürich

Redactor: H. MISLIN, Basel-Mainz

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L'EXPERIENTIA paraît le 15 de chaque mois. Vente et abonnement dans toutes les librairies suisses et étrangères, ou directement chez l'éditeur. Abonnement pour un an frs. 68.-. Ce prix s'entend en francs suisses.

Die EXPERIENTIA erscheint am 15. jedes Monats und kann im In- und Ausland durch jede Buchhandlung oder direkt vom Verlag bezogen werden. Der Abonnementspreis beträgt Fr. 68.-.

Insertionspreise: $\frac{1}{1}$ Seite Fr. 220.-, $\frac{1}{2}$ Seite Fr. 132.-, $\frac{1}{4}$ Seite Fr. 77.-. Inseratenannahme durch den Verlag.

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EXPERIENTIA is published on the 15th of every month and can be obtained in any country through the booksellers or from the publishers. The price by annual subscription is fr. 68.-. Price in Swisscurrency.

Prices for advertising: $\frac{1}{1}$ page fr. 220.-, $\frac{1}{2}$ page fr. 132.-, $\frac{1}{4}$ page fr. 77.-. Advertisements should be sent to the publishers.

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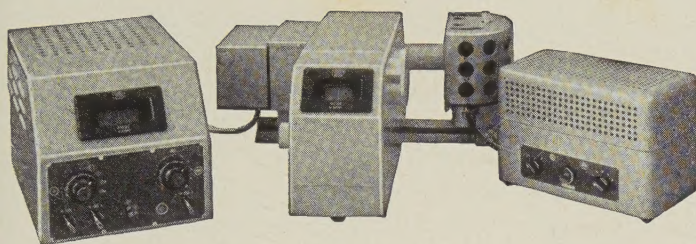
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Endocrine Control of Sexual Behavior in Mammals¹

By A. ANTHONY²

The purpose of this report is to outline the present state of knowledge regarding the role of endocrines in controlling sexual behavior in mammals. It is apparent that any discussion of hormones and behavior must include not only direct but indirect actions of hormones, i.e. through their effects on metabolism, behavioral development, or modification of cognitive receptor pathways. An appreciation of the complexity of the regulatory factors underlying sexual expression can be obtained from the Figure which summarizes some of the interactions between endocrine, experiential, and perceptual factors. In the following review the major lines of evidence supporting the neuro-endocrine basis of sexual behavior will be briefly summarized.

Endocrine control of neural organization underlying sexual behavior. It is logical to consider first the evidence which supports the possibility that the action of maternal or fetal hormones during gestation may have a role in determining sexual behavior at an early stage in development. It is known, for example, that some maternal hormones can cross the placental barrier. Injections of androgens and estrogens into pregnant rabbits, for example, have been shown to produce fetal intersexuality. The action of fetal hormones as important regulators of gonadal development is now fairly well established. In view of the fact that neural and reproductive differentiation occurs concomitantly, it would be reasonable to expect that fetal hormones also influence neural components of sexual behavior during embryonic development. Although this forms our rationale for believing that hormones affect neural organization, there is little evidence to substantiate this. The reason is that no quantifiable criterion of neural change is presently known through which neural-hormonal interactions can be assessed. EAYRS³ has recently reviewed the literature available on hormones and neural maturation and has concluded that the fragmentary evidence 'does little more than suggest the lines of approach to future work'.

Most of the work on fetal hormones has been done on the following mammals by investigators from different laboratories: the opossum by MOORE⁴, the rat by WELLS⁵, the mouse by RAYNAUD⁶, the rabbit by JOST⁷, and the guinea pig by DANTCHAKOFF⁸. All of these workers were primarily concerned with gonadal development rather than behavior.

Recently it has been shown that certain aspects of sexual behavior of adult guinea pigs can be modified through prenatal and postnatal administration of gonadal hormones⁹. For the most part, however, the degree to which adult sexual behavior depends upon hormones circulating in the fetus remains to be studied.

Role of sex hormones and experience as determinants of sex behavior. Let us now consider the problem of hormone-behavior relationship after reproductive maturity where much more information is available. The direct dependence of mating activity on the sex hormones has long been a subject of intensive study, and there is now no doubt that sex hormones play a cardinal role in the control of adult sexual behavior in most mammalian species. The exact mechanism of hormone action, however, remains obscure. Recently, emphasis seems to have turned from the role of hormones, to studies of the functional role of experience, in sexual behavior regulation. It was found that in some species, experience plays an equal, if not more important, role in the facilitation of sexual behavior than do the sex hormones.

Most of what is known regarding the action of hormonal or experiential 'determinants' of sex behavior has come from two broad areas of research: (1) studies of the normal physiology of reproduction in different mammalian species; (2) studies of the effects of the administration of pituitary gonadotrophins or gonadal hormones to immature and mature individuals.

⁴ C. R. MOORE, *Embryonic Sex Hormones and Sexual Differentiation* (Thomas, Springfield, Ill. 1947).

⁵ L. J. WELLS, *Arch. Anat. micr. Morph. exp.* 39, 499 (1950).

⁶ A. RAYNAUD, *Modification expérimentale de la différenciation sexuelle des embryons de souris, par action des hormones androgènes et estrogènes* (Hermann et Cie, Paris 1942); *Arch. Anat. micr. Morph. exp.* 39, 518 (1950); *R. Soc. Biol.* 127, 993 (1938); *Bull. Biol. France Belgique* 72, 297 (1938).

⁷ A. JOST, *Rec. Progr. Hormone Res.* 8, 379 (1953).

⁸ V. DANTCHAKOFF, *C. R. Soc. Biol.* 127, 1255, 1262 (1938); 141, 114 (1947).

⁹ R. W. GOY, C. H. PHOENIX, and W. C. YOUNG, *Anat. Rec.* 131, 559 (1938). - C. H. PHOENIX, R. W. GOY, A. A. GERRALL, and W. C. YOUNG, *Anat. Rec.* 131, 589 (1938).

¹ Presented before the symposium on sexual behavior sponsored by the section of Animal Behavior and Sociobiology of the Ecological Society at the 1957 Meeting of the AAAS. Paper No. 2254 in the journal series of the Pennsylvania Experiment Station.

² Department of Zoology and Entomology, Pennsylvania State University, University Park (Penn.).

³ J. T. EAYRS, *Ciba Found. Coll. Endocr.* 3, 18 (1952); *Brit. J. Animal Behav.* 1 (4), 144 (1953).

The hypothesis currently advanced regarding the neuro-endocrine basis of sexual behavior is summarized in the succeeding paragraphs. Earlier experimental work which forms the basis for this hypothesis was summarized several years ago by BEACH¹⁰.

First, to the best of our knowledge, *the basic neural components of sexual behavior are considered innate*. There is ample experimental documentation showing that sex-specific patterns of neural organization are already present at birth. Since male or female behavior can be elicited by the administration of the proper sex hormone shortly after birth, it must be assumed that the neural organization underlying sexual behavior is laid down independently of the action of sex hormones.

Second, among the factors known to influence the character of sexual behavior, *the gonadal hormones and experience play cardinal roles as determinants of mating activity in most, if not all, vertebrate species*. Relevant in this respect is the fact that the sex hormones do not exhibit absolute specificity. That is, although male and female hormones preferentially facilitate the normal mating behavior of their homotypic sex, each hormone can also facilitate the expression of sex behavior components of the opposite sex to a certain degree. It is important to emphasize that hormones are not responsible for the type of sex behavior of the organism; this presumably has an hereditary basis. The function of the sex hormones may be thought of as analogous to that of a 'film developer', i.e., merely acting to bring to expression an already predetermined innate behavioral repertoire.

Prior to 1942 there was a general tendency to relate differences in patterns of sexual activity to differences in levels of gonadal function. The present belief is that *sex hormones do not necessarily determine the level of sex drive*, although there is still some controversy on this. At any rate, in some species, such as the guinea pig, it appears that the level of the sex drive may largely be a function of the individual's genetic endowment. Exactly how sex hormones act to facilitate sexual expression, or how they establish the level of sex drive, remains a mystery. For the present we are forced to content ourselves with viewing these actions as threshold effects as suggested by GOLDSTEIN¹¹. He postulated that gonadal hormones may have both a specific and a general effect on behavior, i.e., androgens may be thought as primarily acting to facilitate male behavior, and estrogens, female behavior, whereas both hormones also exert general non-specific facilitating effects on both sex patterns in the same animal.

From the limited number of mammalian species which have been studied, it would appear that certain

components of sex behavior are common to most, or all, mammals. These include such activities as recognition and foreplay by both the male and female, lordosis, or 'presentation' by the female, and mounting, intromission, and ejaculation by the male. On the other hand, *considerable inter- and intra-specific variability may exist in the overall mating pattern*. So much so, even within closely inbred strains, that it is a common practice to use each animal as its own control in the study of endocrine-behavior interactions. That is to say, before one can measure the effect of a hormone on the sexual behavior of an animal, he must first establish the normal sex behavior level before treatment has been instituted. Some of the causes of variations in sex behavior of different species may be accounted for on the basis of: (1) differences in the degree to which the pre-determined sexual pattern is genetically established; (2) the relative importance of hormonal versus experimental factors regulating the completeness of frequency of normal mating activities; and (3) the temporal parameters of hormonal and experimental action. Although systematic studies of the time span over which sex hormones exert their actions have just begun, it may be forecast that this area of research will prove to be most fruitful.

There is no doubt that one of the most important contributions to our understanding of the role of endocrines in sex behavior was the demonstration that somatic factors determine or limit the nature of the physiological response to gonadal hormones¹². Equally important was the relatively recent finding that experience in some species plays a much more important role than hormones in the final organization of sex behavior patterns^{11, 12}.

In a recent report, YOUNG cited evidence that socially reared guinea pigs have a higher sex drive than littermates raised in isolation¹². He further reported that the exact time at which the young are isolated from their companions may prove critical in some strains. Little difference was found in the sexual performance of isolated and socially raised males if isolation was instituted on day 25 postnatally. On the other hand, a marked reduction in sexual performance was obtained when males were weaned and isolated on day 10 instead of day 25. In earlier work, GRUNT and YOUNG¹³ and also RISS *et al.*¹⁴ found that although male hormone can restore sex behavior of castrate guinea pigs to preoperative levels, androgen injections were not found capable of augmenting sex behavior beyond the precastrate level. These workers reported that contact with other animals has an organizing

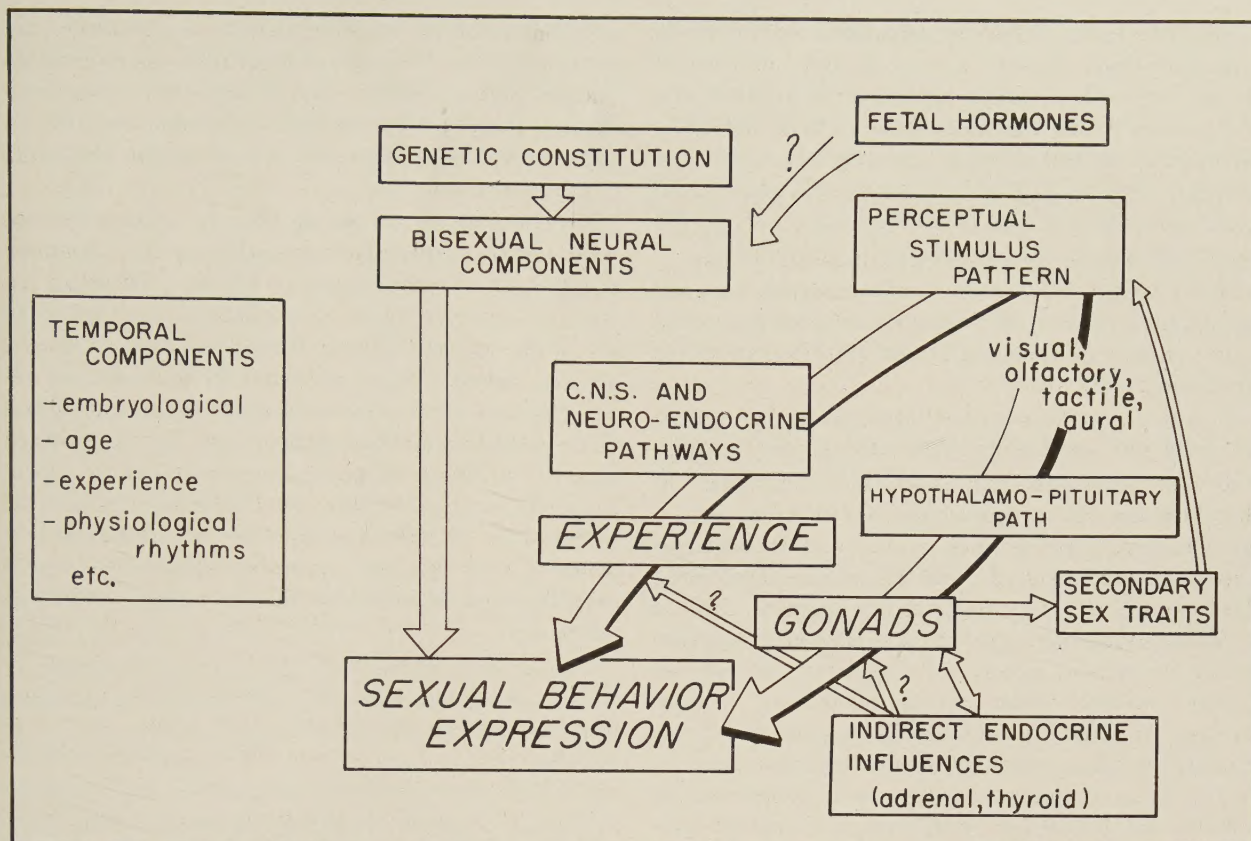
¹⁰ F. A. BEACH, *Rec. Progr. Hormone Res.* 1, 27 (1947); *Physiol. Rev.* 27, 240-306 (1947); *Hormones and Behavior* (Paul B. Hoeber, Inc., New York and London 1948).

¹¹ A. C. GOLDSTEIN, in *Hormones, Brain Function, and Behavior* (Acad. Press, N. Y. 1957), p. 99.

¹² W. C. YOUNG, in *Hormones, Brain Function, and Behavior* (Acad. Press, N. Y. 1957), p. 75.

¹³ J. A. GRUNT and W. C. YOUNG, *J. comp. Physiol. Psychol.* 46, 138 (1953).

¹⁴ W. RISS, E. S. VALENSTEIN, J. SINKS, and W. C. YOUNG, *Endocr.* 57 (2), 139 (1955). - E. S. VALENSTEIN, W. RISS, and W. C. YOUNG, *J. comp. Physiol. Psychol.* 48 (5), 397 (1955).



Regulatory mechanisms influencing sex behavior.

action on the development of the copulatory pattern in the male guinea pig. They also found that genetic differences in the level of sexual excitement are not overcome by large doses of androgen¹⁴.

In the experiments using rats, however, BEACH and HOLZ-TUCKER¹⁵ have reported that in the castrate rat, androgens not only restore mating behavior to the pre-operative level but also cause an improvement in certain measures of sex behavior, if the maintenance dose of male hormone is exceeded. Recently, BEACH found no differences in the percentage of isolated and socially reared rats exhibiting completely organized sexual patterns on the first behavioral trial¹⁶. This indicates that, at least in the rat, experience is not essential for sexual behavior. On the other hand, ROSENBLATT and ARONSON report that in the male cat the mating pattern depends upon the combined influence of sexual experience and high androgen levels¹⁷.

There are other differences in hormone-behavior regulatory mechanisms of the guinea pig and the rat. YOUNG¹⁸ found that the mounting occurs only at estrus

in female guinea pigs and is abolished by ovariectomy. BEACH¹⁹ reports that in female rats, mounting occurs throughout the entire estrous cycle and persists after ovariectomy. Paradoxical findings such as these point to the need for more experimental work on behavioral effects of hormones in different mammalian species.

Appropriate in this regard are a number of studies dealing with the effects of experience and hormones in the domestic fowl²⁰. Most of the reported findings support the belief that the degree of social experience affects the level of sexual expression in birds. On the basis of their current work with turkeys, however, SCHEIN and HALE state that transient test conditions rather than differential social and sexual experience may contribute to the apparent differences in levels of response²¹. From their work on turkeys they believe that levels of sexual expression are not dependent upon the degree of early social experience. One must conclude that the problem of the relative importance of experience and hormones in controlling sexual behavior is still not completely resolved for either mammals or birds.

Apart from the known dependence of the neuro-humorally regulated ovulatory response on coitus in the

¹⁵ F. A. BEACH and A. M. HOLZ-TUCKER, *J. comp. Physiol. Psychol.* **42**, 433 (1949).

¹⁶ F. A. BEACH, *J. comp. Physiol. Psychol.* **51**, 37 (1958).

¹⁷ J. S. ROSENBLATT and L. R. ARONSON, *Anim. Behav.* **6**, 171 (1958).

¹⁸ W. C. YOUNG, E. W. DEMPSEY, C. W. HAGQUIST, and J. L. BOLING, *J. comp. Psychol.* **27**, 49 (1939).

¹⁹ F. A. BEACH, *Endocr.* **31**, 673 (1942).

²⁰ D. G. M. WOOD-GUSH, *Anim. Behav.* **6**, 68 (1958); *Proc. R. physiol. Soc. Edinburg* **27**, 6 (1958).

²¹ M. W. SCHEIN and E. B. HALE, *Anat. Rec.* **128**, 617 (1957); *Poult. Sci.* **37**, 1240 (1958); unpublished data.

cat, rabbit, and ferret, there is no information presently available which directly relates to the influence of animal interactions during mating on the endocrines. There is no doubt that during the sexual encounter there occur marked shifts in the secretory activity of such glands as the adrenal and thyroid. To what extent sexual behavior is in turn modified by the nature of the endocrine response still remains to be investigated.

Indirect endocrine influence on sex behavior. BEACH²² has pointed out that all hormones which affect metabolic processes of tissues will exert some indirect effect on sex behavior.

Because of the close interactions between the adrenals and gonads²³, and the thyroids and gonads²⁴, adrenocortical and thyroid secretions have long been suspected of influencing the responsiveness of tissues mediating sex behavior. There is considerable evidence that *abnormal* thyroid or adrenal function may have profound effects on sexual behavior. Thus far, however, there is little direct evidence bearing on the problem of whether sexual behavior is closely correlated with adrenal or thyroid function under normal conditions. Several attempts to define the nature of thyroid effects on sexual performance have not met with much success. YOUNG²⁵ reports that sexual behavior in the guinea pig is relatively unaffected by injections of thyroid hormone, or by thyroidectomy; HEIDENREICH²⁶ obtained similar results using rats. WARREN and ARONSON²⁷ found that prepuberal castration of male hamsters does not prevent the development of some aspects of the sexual pattern. These workers also found that the behavior of the hamster that is both castrated and

adrenalectomized before maturity is essentially the same as that of the simple castrate. It should be pointed out, however, that except for a few such studies there are no quantitative data available on behavioral changes as a function of changes in thyroid or adrenal activity.

One generalization which can be drawn is that sexual performance is dependent upon the adequate functioning of many organs and tissues. The most important endocrine organ to which has been delegated the duty of maintaining tissue homeostasis is the adrenal cortex. Since adrenal activity may be influenced by a variety of conditions including nutritional state, temperature fluctuations, age, and even daily activity rhythms, it would be reasonable to expect some relationship between adrenal activity and sexual performance. Unfortunately, there is insufficient evidence to even speculate as to the degree that sexual responsiveness may depend on such indirect endocrine influences.

There would appear to be important advantages of working on the hormone-behavior problem in seasonal breeders. An experimental analysis of the seasonally breeding prairie dog by ANTHONY²⁸ showed that there was a close correspondence between increased activity of animals, changes in adrenal function, and the onset of breeding, indicating that adrenal influence may have an important role in regulating mating behavior. ANTHONY²⁹ also observed that cage confinement inhibits mating behavior, despite the fact that there is no observable effect on gonadal development, which supports the belief that the sex hormones are not the sole regulators of sexual behavior in some species.

Résumé

L'auteur présente une revue critique des bases expérimentales de l'hypothèse neuro-endocrine du comportement sexuel des mammifères.

²² F. A. BEACH, in *Handbook of Experimental Psychology* (John Wiley and Sons, New York 1951), p. 387.

²³ A. S. PARKES, *Physiol. Rev.* 25 (2), 203 (1945).

²⁴ M. MAQSOOD, *Biol. Rev.* 27 (3), 281 (1952).

²⁵ W. C. YOUNG, B. RAYNOR, R. R. PETERSON, and M. M. BROWN, *Endocr.* 51 (1), 12 (1952).

²⁶ W. F. HEIDENREICH, C. E. ALEXANDER, and F. A. BEACH, *Endocr.* 52 (6), 719 (1953).

²⁷ R. P. WARREN and L. R. ARONSON, *Endocr.* 58, 293 (1956); *J. comp. Cell. Psychol.* 50 (5) 475 (1957).

²⁸ A. ANTHONY, *J. Morph.* 93 (2), 331 (1953).

²⁹ A. ANTHONY, *J. Mamm.* 36 (1), 69 (1955).

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High Pressure Preparation and Structure of Crystalline Nickelous Carbonate**

LANGLÈS¹ recently succeeded in preparing crystalline NiCO₃ for the first time. He obtained two forms, de-

pending on the reagents used and the conditions of precipitation. The two forms possessed somewhat different lattice constants, but belonged to the same space group. Consequently, a polymorphic transition is unlikely. It is possible that his material might have been of slightly inferior purity.

In the present study colloidal NiCO₃ was precipitated from aqueous solutions of nickelous sulfate and sodium

** Publication No. 148 of the Institute of Geophysics.

¹ R. DE S. L. LANGLÈS, *Ann. Chim., Paris* [12] 7, 568 (1952).

Powder data

$d_{\text{obs.}}$ in Å	$d_{\text{calc.}}$ in Å	hk. l	100 I/I ₀
3.504	3.506	01.2	42
2.704	2.706	10.4	100
2.299	2.301	11.0	20
2.089	2.085	11.3	26
1.922	1.924	20.2	18
1.752	1.753	02.4	7
1.676	1.680	11.6	28
1.477	1.476	12.2	15
1.380	1.383	10.10	20
1.354	1.353	20.8	5
1.329	1.328	30.0	6
1.226	1.229	00.12	4
1.187	1.185	02.10	2
1.165	1.166	12.8	4
	1.168	30.6	
1.149	1.150	22.0	3

bicarbonate. The colloidal material was thoroughly washed and dried. It contained less than 0.005% Pb, 0.001% Fe, 0.01% Co, and 0.005% Cu.

It proved to be impossible to secure crystallization by heating the colloidal NiCO_3 up to 10 h in an atmosphere of CO_2 (1 bar pressure) at temperatures ranging between 100°C and 300°C.

The colloidal material was then subjected to a pressure of 20,000 bars and a temperature of 500°C in the 'simple squeezer' high-pressure apparatus developed by GRIGGS and KENNEDY². 15 min under these conditions was sufficient to allow complete crystallization to take place. It is, however, probable that crystallization will take place under much less extreme conditions of temperature and pressure, but the 'simple squeezer' press was available and proved to be quite satisfactory for the purpose, although a small amount of NiO was formed at the same time, due to partial loss of CO_2 from the system.

The resulting green material was finely ground, and the X-ray powder diffraction pattern at 25°C was obtained by means of a Norelco High Angle recording diffractometer, using CuK_α radiation ($\lambda = 1.5418$ Å) and a Ni filter. Pre-calibrated sodium chloride was used as an internal standard. The results are given in the Table.

It was found that NiCO_3 crystallizes in the rhombohedral sub-group of the hexagonal system. Systematic extinctions appeared to be the same as for calcite, and it is consequently assumed that the space group is also the same, viz. $R\bar{3}c$.

The hexagonal unit-cell dimensions at 25°C, obtained by means of a least-squares method, are:

$$\begin{aligned}a_0 &= 4.602 \pm 0.001 \text{ Å} \\c_0 &= 14.744 \pm 0.003 \text{ Å},\end{aligned}$$

yielding an axial ratio

$$c_0/a_0 = 3.204.$$

The dimensions of the corresponding rhombohedral unit-cell are:

$$\begin{aligned}a_{\text{rh}} &= 5.587 \text{ Å} \\ \alpha &= 48^\circ 38' .\end{aligned}$$

² D. T. GRIGGS and G. C. KENNEDY, Amer. J. Sci. 254, 722 (1956).

This may be compared with the results of LANGLE¹, who found:

For yellow NiCO_3 : $a_{\text{rh}} = 5.60$ Å, $\alpha = 48^\circ 31'$.

For green NiCO_3 : $a_{\text{rh}} = 5.57$ Å, $\alpha = 48^\circ 43'$.

The present values of a_{rh} and α are almost equidistant from the two sets of earlier values. It appears reasonable to conclude that the 'yellow' and 'green' NiCO_3 of LANGLE are identical.

Assuming a rhombohedral unit-cell containing 2 molecules, the density of NiCO_3 at 25°C is 4.371 g/cm³.

The crystals were too tiny to determine the refractive indices under the petrographic microscope.

C. W. F. T. PISTORIUS*

Institute of Geophysics, University of California, Los Angeles, May 5, 1959.

Zusammenfassung

Kristallines NiCO_3 wurde durch Behandlung des nicht-kristallinen Stoffes unter hohem Druck (20 000 bar) bei 500°C gewonnen. Die rhomboedrische Einheitszelle (Raumgruppe $R\bar{3}c$) besitzt die folgenden Dimensionen:

$$a_{\text{rh}} = 5.587 \text{ Å}; \quad \alpha = 48^\circ 38'.$$

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On the Contamination of the Murphy-Sturm Lymphosarcoma with a Pleuropneumonia-like Organism¹

An experimental polyarthritis can be readily produced in rats by the intraperitoneal injection of various tumor exudates^{2,3}. Cultivation of the Murphy-Sturm lymphosarcoma exudate—one of the most active in this respect—revealed that a pleuropneumonia-like organism of the L4 strain was the causative agent^{4,5}.

This report describes subsequent observations arising from experiments performed in an attempt to: (a) decontaminate the Murphy-Sturm lymphosarcoma; (b) propagate the infection to other transplantable tumors; (c) evaluate the resistance to arthritis in animals implanted with tumors liable or not liable to produce articular lesions; (d) test the activity of the exudate from a Murphy-Sturm lymphosarcoma grown in another laboratory.

Methods

Female Sprague Dawley rats weighing between 140 and 170 g were used in these experiments. They were kept on Purina Fox Chow and received tap water *ad libitum*. Specifications of group arrangements and modality of

¹ This work was supported by a grant from the National Cancer Institute of Canada. The authors also gratefully acknowledge generous gifts of terramycin from Pfizer Canada, Montreal, and neomycin from The Upjohn Co., Kalamazoo, U.S.A.

² G. JASMIN, Rev. canad. Biol. 15, 107 (1956).

³ C. L. RICHER and G. JASMIN, Rev. belge Path. Méd. exp. 26, 94 (1957).

⁴ G. JASMIN, Ann. rheum. Dis. 16, 365 (1957).

⁵ E. KLIENEGER-NOBEL, Personal communication (1956).

Susceptibility to Arthritis of Rats Implanted with a Tumor and Injected with a Tumor Exudate

Groups	Tumors	Exudates	Arthritic Response*	
			Incidence	Severity
I	None	Murphy-Sturm lymphosarcoma	8	+++
		Croton oil fibrosarcoma	7	++
II	Murphy-Sturm lymphosarcoma .	Murphy-Sturm lymphosarcoma	0	0
		Croton oil fibrosarcoma	6	+
III	Croton oil fibrosarcoma	Murphy-Sturm lymphosarcoma	7	++
		Croton oil fibrosarcoma	0	0
IV	Yoshida sarcoma	Murphy-Sturm lymphosarcoma	8	+++
		Croton oil fibrosarcoma	8	+++

* Each sub-group consisted of 8 rats.

treatment are outlined in the appropriate sections of results.

The tumor exudates are prepared as follows: A neoplastic pouch is produced by injecting 0.5 ml of tumor suspension (1 g of freshly ground tumor in 5 ml of a 1% saline solution) into a 25 ml air space in the connective tissue under the dorsal skin of the rat. As the tumor grows, the cavity fills with exudate and on the tenth day, this liquid is withdrawn with an 18 gauge needle and centrifuged (200 r.p.m. for 20 min) before being used for experimental purposes.

Whenever tumors are transplanted subcutaneously, a cellular suspension is prepared as previously described and is injected in the same region without previous inflation of the skin. In the case of the croton oil fibrosarcoma⁶, however, the transplantation was performed by inserting slices 2 cm × 1 cm × 3 mm in size, under the dorsal skin through a small incision that was closed with two sutures.

The arthritic reaction in rats was appraised visually and our readings were recorded according to an arbitrary scale of 0 to +++ between the fourth and the sixth day following intraperitoneal injection of 1 ml of centrifuged exudate.

Results

Experiment I: *Attempts to decontaminate the Murphy-Sturm lymphosarcoma.*—Preliminary trials have shown that application of heat, ultraviolet light or antiseptics succeeds in sterilizing a tumor suspension, but not without affecting the cellular integrity that is indispensable for transmission of the neoplasm. Therefore, two antibiotics, terramycin and neomycin, were used, because of their relative innocuity to the tumor cells and their known efficacy against pleuropneumonia-like organisms. Each antibiotic was administered to a group of four rats, first by adding 400 γ to the tumor suspension used for implantation and, then, by subcutaneous injection of 2.5 mg, twice daily. The treatment lasted for ten days after which time the exudate was withdrawn and tested for its arthritic activity. The tumor was then transferred to another host similarly treated with either one of the antibiotics.

Although this procedure was repeated 12 times consecutively, it was impossible to decontaminate the tumor as judged by the sustained activity of the exudate to produce arthritis. However, we noted that the tumor growth and exudation were slightly reduced by the antibiotic therapy, although on the other hand, no necrotic tissue was found in the pouches of these animals.

Experiment II: *Inoculation of other transplantable tumors.*—In order to determine whether the polyarthritic

infective agent is transmissible, three different tumors implanted into pouches were inoculated with a small volume (0.1 ml) of Murphy-Sturm lymphosarcoma exudate. The pouches were made in a series of 24 rats divided into three equal groups transplanted with Yoshida sarcoma, hepatoma 130 or 7974, respectively. Each group was then subdivided so that four animals were used as controls and the four others were inoculated with centrifuged Murphy-Sturm lymphosarcoma exudate into the pouch, five days after transplantation of the tumor.

All exudates taken from the inoculated tumor pouches showed a high arthritogenic effect. The three contaminated tumors were then re-implanted in new hosts and their exudates still produced arthritis. However, following the fifth passage the tumors regressed and could no longer be propagated.

Experiment III: *Arthritic reaction in tumor bearing animals.*—Sixty-four rats were divided into four equal groups. The first group served as controls and the three others were implanted with Murphy-Sturm lymphosarcoma, croton oil fibrosarcoma or Yoshida sarcoma, respectively, underneath the skin of the back. Eight days after implantation, the original groups were subdivided: eight rats were injected intraperitoneally with Murphy-Sturm lymphosarcoma exudate and eight rats, with exudate from the croton oil fibrosarcoma, as shown in the Table.

Both exudates elicited an arthritic reaction in normal animals. Rats bearing a Murphy-Sturm lymphosarcoma and croton oil fibrosarcoma were found resistant to their homologous exudate only. Such resistance was not demonstrable after cross treatment with these same exudates. However, the arthritic response to exudate from the croton oil fibrosarcoma was lowered in rats implanted with the Murphy-Sturm lymphosarcoma. On the other hand, the Yoshida sarcoma, which is devoid of arthritogenic properties, conferred no protection.

Experiment IV: *Arthritogenic action of a Murphy-Sturm lymphosarcoma grown in another laboratory.*—Using a Murphy-Sturm lymphosarcoma that was supplied to us by another research laboratory, it was possible to reproduce the arthritic reaction by injecting either a tumor pouch exudate or an autolyzed tumor cell suspension (kept for 48 h at room temperature). The polyarticular lesions occurred within six days in two groups of ten normal rats injected with 1 ml of either tumor fluid, respectively.

Discussion

The exudates of the Murphy-Sturm lymphosarcoma grown in two different laboratories produce an infectious polyarthritis, as a result of their being contaminated with a pleuropneumonia-like organism. To our knowledge, this contaminating agent has not yet been reported by other

⁶ This tumor was also named Croton-Pouch Tumor No. 17.
⁷ H. SELYE, J. nat. Cancer Inst. 15, 1291 (1955).

workers, although the tumor has been the object of several investigations. In our experience, two other transplantable tumors, the Novikoff hepatoma and the croton oil fibrosarcoma, exhibit similar infective properties^{2,3}. Strains of pleuropneumonia-like organisms were also isolated from HeLa cells grown in various laboratories⁸. Thus, it appears that contamination of neoplastic tissue with this particular microorganism is a common feature.

Our unsuccessful attempts to decontaminate the Murphy-Sturm lymphosarcoma with terramycin and neomycin would suggest that the microorganism is highly incorporated in the tumor cells. On the other hand, inoculation of other tumors with Murphy-Sturm lymphosarcoma exudate revealed that the contaminant does not live in symbiosis with all types of tumor cells. It even causes tumor regression, a finding that most probably explains the antitumor properties of a Murphy-Sturm lymphosarcoma autolysate previously demonstrated by one of us⁹.

The fact that protection against arthritis can be evidenced only in animals implanted with a tumor homologous to the exudate is puzzling. It seems most probable that we are dealing with two different strains of pleuropneumonia-like organisms, each having its specific immune reaction. The particular affinity of these microorganisms for tumor tissue deserves further investigation, and their presence should always be suspected when results arising from experiments performed with a transplantable tumor are being interpreted.

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Departments of Pathological Anatomy, Histology, and Embryology, University of Montreal (Canada), April 27, 1959.

Résumé

La terramycine et la néomycine ne parviennent pas à décontaminer le lymphosarcome de Murphy-Sturm infecté par un «pleuropneumonia-like organism». Il a été constaté que ce microorganisme est transmissible à d'autres tumeurs transplantables. Le rat porteur d'une tumeur infectée devient résistant à l'induction d'arthrite par l'exsudat d'une tumeur homologue. L'exsudat d'un lymphosarcome de Murphy-Sturm provenant d'un autre laboratoire est également doué de propriétés arthritogènes.

⁸ L. H. COLLIER, *Nature* 180, 757 (1957).

⁹ C. L. RICHER, *Proc. Soc. exp. Biol. Med.*, N.Y. 96, 548 (1957).

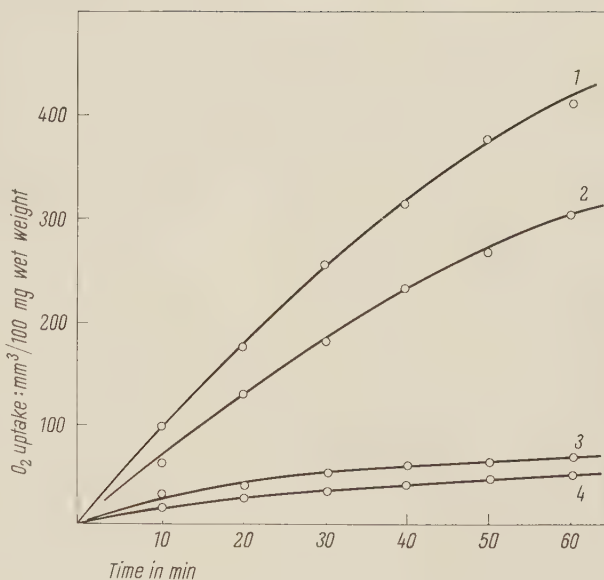
* Medical Research Associate of the National Research Council of Canada.

Hepatic Cyclophorase Activity in Rats Treated with 1,2,3,4-Tetrahydro- β -naphthylamine (THNA)

Previous experiments carried out in our laboratory showed that administration of THNA (a powerful pyrogenic agent which causes a rise in the basal metabolic rate and respiration) produces a great fall in liver and muscle glycogen content of the rat¹ and increased *in vivo* acetylation of para-aminobenzoic acid (PABA)². Since 'active acetate' (acetyl-Coenzyme A) can be generated from sugars via pyruvic acid, a probable way by which carbohydrates could be metabolized at a faster rate was by an increased oxidation to CO₂.

¹ V. MARTINI and M. ORUNESU, *Boll. Soc. Ital. Biol. sperim.* 33, 1682 (1957).

² V. MARTINI and M. ORUNESU, *Boll. Soc. Ital. Biol. sperim.* 33, 1684 (1957).



Endogenous respiration and pyruvate oxidation by liver homogenates from THNA-treated rats and controls

Each point on the curves represents the mean value of 12 experiments. The rates of O₂ uptake are expressed per 100 mg of wet weight of liver tissue present in the homogenate. Each Warburg flask contained: 0.5 ml of 10% (W/V) liver homogenate; 0.3 ml of 0.1 M phosphate buffer (pH 7.6); 0.3 ml of 0.01 M Na-ATP; 0.4 ml of 0.03 M MgCl₂; 0.2 ml of 0.1 M Na-pyruvate; 0.2 ml of 0.1 M Na-fumarate and 1.1 ml of 0.12 M KCl-0.02 M NaHCO₃ solution. 0.2 ml of 15% KOH was placed in the center well. Each vessel contained 50 ± 2.2 (s.d.m.) mg of fresh liver tissue. Total volume: 3.2 ml. Gas phase: air. Temperature: 38°C. The flasks were allowed to equilibrate for 7 min and readings were taken at 10 min intervals without interrupting the shaking. Duplicate estimations were made in all cases. — Curves 1 and 2: oxidation of pyruvate by liver homogenates from treated and control rats respectively; curves 3 and 4: endogenous respiration of homogenates from treated and control rats.

The experiments described below were undertaken to investigate whether or not, in THNA-treated rats, liver glycogen depletion could depend on Krebs Cycle operating at a faster rate.

Material and methods.—Male albino rats from a local strain weighing 180–230 g were used throughout. All rats were fasted for 12 h before being sacrificed. Four animals for each experiment were used: two rats were given a single intraperitoneal injection of THNA dissolved in physiological saline (3 mg/100 g of body weight) and two served as controls. 4 h later, the rats were killed by a blow on the head and exsanguination. The excised, bled liver was rapidly ground in a Potter-Elvehjem all glass apparatus with nine parts by weight of 0.12 M KCl–0.02 M NaHCO₃ cold solution at pH 7.6. The homogenates were used immediately for measurement of enzyme activity.

Oxygen absorption was measured by the direct manometric method of Warburg. In evaluating the data, mean values obtained from treated rats have been compared with those of control groups and designated significantly different only when the probability value, using the 't' test, was equal to or less than 0.02³.

Results

(1) *Endogenous oxygen uptake and oxidation of pyruvate by liver homogenates from rats treated with THNA and controls.*—As shown in the Figure, the oxygen consumption in

³ F. J. MOORE, F. B. CRAMER, and R. G. KNOWLES, *Statistics for Medical Students* (The Blakiston Company, Toronto 1951).

Table

Oxidative activities of liver homogenates from THNA-treated rats and controls

The experimental conditions are the same as those given in the Figure. The final concentration of the added substrates was 0.02 M. The rates of O_2 uptake are expressed as $\mu\text{l/h}/100$ mg of wet weight. All values are corrected for endogenous respiration and represent the average of the number of the experiments presented. The significance of the difference between means is indicated by the value of 'P'.

No. of rats	Experimental condition	Substrate added	O_2 uptake mean	Value of 'P'	% Increase in O_2 uptake
6	Controls	Na-citrate	85	< 0.02	
6	THNA-treated	Na-citrate	112		32
12	Controls	Na-succinate	263	= 0.01	
12	THNA-treated	Na-succinate	358		36
4	Controls	Na-fumarate	104	< 0.02	
4	THNA-treated	Na-fumarate	132		27
6	Controls	Na-malate	91	< 0.02	
6	THNA-treated	Na-malate	116		28
8	Controls	Na-glutamate	188	= 0.05	
8	THNA-treated	Na-glutamate	215		14

the presence of pyruvate (with fumarate added to furnish a supply of oxalacetate for the condensation reaction which causes citrate formation) is considerably higher when liver homogenates from treated rats are employed.

Endogenous oxygen uptake by liver homogenates from THNA-treated rats shows a slight and somewhat erratic enhancement in comparison with controls. Only in 6 experiments out of 15, a marked increase in endogenous respiration was observed, but its extent never reached that found when pyruvate and fumarate were added.

(2) *Oxidation of some Krebs Cycle intermediates by liver homogenates from THNA-treated rats.* In the sets of experiments shown in the Table, liver homogenates from normal and THNA-treated rats were incubated with some Citric Acid Cycle intermediates.

The added substrates were oxidized at very different rates, however, it can be seen that THNA-administration resulted in all cases in higher oxygen consumption by liver homogenates. An interesting feature of the above experiments is that, regardless of vast differences in oxygen consumption using different Tricarboxylic Acid Cycle intermediates, the percentage increase in O_2 uptake by liver homogenates from treated rats is essentially the same. The oxidation of glutamate proved to be less affected by THNA treatment than that of the above substrates employed.

Discussion.—If conclusions regarding the *in vivo* effects of THNA can be drawn from experiments carried out under *in vitro* conditions, our findings would suggest that THNA stimulates the hepatic Cyclophorase activity of the rat. Judging from the experiments in which pyruvate and fumarate were employed, the preparations from treated rats appear to produce (and to oxidize) 'active acetate' more readily than controls.

Our experiments do not offer any explanation of the mechanism by which THNA stimulates Krebs Cycle activity; however, it seems of interest to note that the *in vivo* effects of THNA resemble, in some respects, those of 'uncoupling' agents such as 2,4-dinitrophenol, thyroxine and triiodo-thyronine. One could therefore put forward the hypothesis that THNA causes an acceleration of the oxidative processes at the expense of chemical efficiency in generating high-energy phosphate bonds required in the anabolism (synthesis) of carbohydrates. Work to study the *in vivo* and *in vitro* effects of THNA upon oxidative phosphorylation, and to control whether the above hypothesis is justified, is now under progress and will form the subject matter of subsequent communications.

In conclusion we admit that our results lend strong support to assume that THNA administration to rats brings about enhanced carbohydrate breakdown by stimulating Krebs Cycle activity.

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Institute of General Physiology, University of Bari (Italy), April 13, 1959.

Riassunto

Gli omogenati di fegato dei ratti sacrificati dopo 4 h dalla somministrazione di β -tetraidronaftilammina (30 mg pro kg per via intraperitoneale) presentano una aumentata capacità di ossidare alcuni composti del Ciclo di Krebs, in rapporto ai controlli. Viene avanzata l'ipotesi che l'intensa glicogenolisi epatica e muscolare e l'aumentata acetilazione *in vivo* dell'acido para-aminobenzoico provocate nel ratto dalla somministrazione di β -tetraidronaftilammina, siano causate da un esaltato metabolismo intermedio ossidativo.

In vitro Effects

of 1,2,3,4-Tetrahydro- β -naphthylamine (THNA) on Succinate Oxidation by Rat Liver Homogenates

Previous experiments^{1,2} showed that THNA, a powerful pyrogenic agent, when added to rat liver homogenates, exerts a slight inhibition on oxidation of malate and significantly increases total O_2 uptake, when succinate is employed as substrate. These *in vitro* effects on succinate and malate oxidation tended to prove that THNA acts through a mechanism similar to that of thyroxine, which has recently been described by WOLFF, CLARKE and BALL^{3,4}, ESTABROOK⁵ and BARKER⁶. These investigators admitted that the increased oxidation of succinate by fresh rat heart homogenates in the presence of thyroxine is due to its capacity of inhibiting the malate dehydrogenase

¹ V. MARTINI and M. ORUNESU, Boll. Soc. Ital. Biol. sperim. **34**, 633 (1958).

² J. TEN CATE and T. KNOPPERS, Arch. Neerl. Physiol. **26**, 352 (1942).

³ E. C. WOLFF and E. G. BALL, J. biol. Chem. **224**, 1083 (1957).

⁴ E. C. CLARKE and E. G. BALL, Fed. Proc. **14**, 193 (1955).

⁵ R. W. ESTABROOK, H. A. NEUFELD, and W. B. MASON, Fed. Proc. **13**, 205 (1954).

⁶ S. B. BARKER, Endocrinology **61**, 534 (1957).

Table I

Effect of THNA on succinate oxidation by 'aged' rat liver homogenates

Time min	mm ³ O ₂ uptake		Value of 'P'	% Variation in the presence of THNA
	Controls	with THNA		
20	31 ± 5.0	41 ± 4.6	< 0.02	+ 32
40	48 ± 3.7	65 ± 4.2	< 0.02	+ 35
60	63 ± 5.5	84 ± 5.8	< 0.02	+ 33

Each Warburg flask contained: 0.5 ml of 'aged' liver homogenate (5% W/V); 0.3 ml of 0.1 M phosphate buffer (pH 7.6); 0.2 ml of 0.01 M Na-ATP; 0.4 ml of 0.003 M MgCl₂; 0.3 ml of 0.05 M Na-succinate; 1.0 ml of 0.12 M KCl-0.02 NaHCO₃ solution; 0.3 ml of 0.01 M THNA or water (controls). 0.2 ml of 15% NaOH in the center well. Duplicate estimations were made in all cases. Gas phase: air. Temperature: 38°C. The flasks were allowed to equilibrate for 7 min and readings were taken at 20-min intervals for 60 min. The rates of O₂ uptake are expressed per 25 mg of wet weight of tissue. Mean values of 14 experiments ± standard deviation after endogenous O₂ uptake subtraction are presented. The significance of the difference between means is indicated by the value of 'P'.

activity and the accumulation of oxalacetate, a strong inhibitor of succinic dehydrogenase.

Some preliminary experiments indicated that the enhancing effect of THNA on succinate oxidation by rat liver homogenates is somewhat different from that of thyroxine and could perhaps be ascribed to a 'direct' stimulation of succinic oxidase system activity. In view of this possibility, the *in vitro* effects of THNA on succinate oxidation by rat liver homogenates were further investigated.

Animals and Methods. Male albino rats from a local strain were used throughout. Liver homogenates were prepared by grinding a portion of the liver in a Potter-Elvehjem all-glass apparatus with cold 0.12 M KCl-0.02 M NaHCO₃ solution to yield a 5% W/V suspension. The homogenates were employed within a 2 h period of 'aging' in order to allow the maximal loss of DPN (Diphosphopyridine Nucleotide) and the consequent inhibition both of malate dehydrogenase activity and oxalacetate formation.

Succinate oxidation was determined manometrically according to the method of AISENBERG and POTTER⁷. Cytochrome oxidase activity was measured by the manometric technique of SCHNEIDER and POTTER⁷. Succinic dehydrogenase activity was followed by observing the time for 90% reduction of Methylene Blue anaerobically⁷. The experimental conditions are given in connection with Tables I to II.

Results and Discussion. The effect of THNA addition on oxygen uptake by rat liver 'aged' homogenates in the presence of succinate is shown in Table I.

The increased rate of O₂ uptake in the presence of 0.001 M THNA was regularly observed from the earlier time intervals and was maintained over prolonged periods of incubation. Since it is well known that succinate oxidation by rat liver fresh homogenates falls off rapidly with time, unless oxalacetate (which inhibits succinic dehydrogenase activity) is removed, the possibility of THNA effects being similar to those already reported for thyroxine was considered: i.e. that a THNA-inhibited formation of oxalacetate might have been responsible for the enhanced O₂ uptake. Succinate oxidation was then

Table II

Effect of THNA on the reduction of methylene blue by rat liver succinic dehydrogenase complex (S. D. C.)

Time min	% reduction of Methylene Blue		% Variation in the presence of THNA
	Controls	with THNA	
3	26	33	+ 27
6	46	58	+ 26
9	64	80	+ 25
12	79	90	+ 14
15	90	90	—

Thunberg tubes especially built to be located into the EEL photo-electric colorimeter were used. The systems consisted of: 1 ml of Na-succinate 0.008 M; 1 ml of Methylene Blue (10 mg%); 1.6 ml of 0.1 M phosphate buffer (pH 7.6) and 0.4 ml of 0.02 M THNA or water (controls). 0.5 ml of liver homogenate was placed into the side arm. The tubes were attached to a manifold and evacuated for 3 min with a high-vacuum pump. The contents of the tube and side arm were mixed after 5 min incubation in a 38°C water bath. At 3 min intervals the tubes were removed, wiped and read in the colorimeter with a 660 mμ filter. The activity was followed by observing the time for 90% reduction of Methylene Blue anaerobically. The values recorded in the Table represent the mean of 20 experiments.

investigated in the presence of glutamate which removes oxalacetate by transamination to form aspartate and α-ketoglutarate, neither of which inhibits the succinic dehydrogenase activity. On a percentage basis, the results obtained were quite similar to those reported in Table I.

These observations focussed our attention on the possibility that the increased oxygen uptake might imply a more specific action of THNA on the succinic oxidase system. According to this assumption, experiments were undertaken to study the enzyme systems bringing about the oxidation of succinate, i.e. succinic dehydrogenase and Cytochrome oxidase. It was observed that THNA addition does not alter significantly the rat liver Cytochrome oxidase activity. In contrast to this, the activity of the succinic dehydrogenase complex (SDC), as judged by the reduction of Methylene blue, is significantly stimulated in the presence of THNA in a final concentration of 0.002 M (Table II).

Thus the results illustrated in Table II might suggest that the increased succinate oxidation in the presence of THNA may be due to a 'direct' stimulation of succinic dehydrogenase activity.

Although the above findings seem to be in agreement with the increased succinate oxidation by liver homogenates from rats previously treated with THNA⁸, they can be considered only as indicative, since the greatest care must be taken in drawing conclusions regarding *in vivo* effects from experiments carried out under *in vitro* conditions.

V. MARTINI and M. ORUNESU

Institute of General Physiology, University of Bari (Italy), April 13, 1959.

Riassunto

Il consumo di ossigeno e la decolorazione del Bleu di Metilene dovuti all'ossidazione del succinato da parte di omogenati di fegato di ratto, appaiono nettamente aumentati in presenza di β-tetraidronaftilammina. Viene prospettata la eventualità che tale sostanza sia in grado di esercitare un'azione attivante «diretta» sul sistema succinato-deidrogenasico.

⁷ W. W. UMBREIT, R. H. BURRIS, and J. F. STAUFFER, *Manometric Techniques* (Minneapolis 1957).

⁸ V. MARTINI and M. ORUNESU, *Exper.* 15, 331 (1959).

Synthesis of a Decapeptide from the Polymyxin Series

During the course of structural studies with the group of polypeptide antibiotics known as polymyxins, HAUSMANN and CRAIG¹ were able to separate a product of polymyxin B into two fractions B₁ and B₂, which differed only in the nature of the fatty acid component. According to their degradation studies, HAUSMANN² and later BISERTE and DAUTREVAUX³ independently proposed for polymyxin B₁ two alternative structures A et B (Fig. 1)⁴ with the only difference that one L-Dab residue was located in the side chain instead of in the ring.

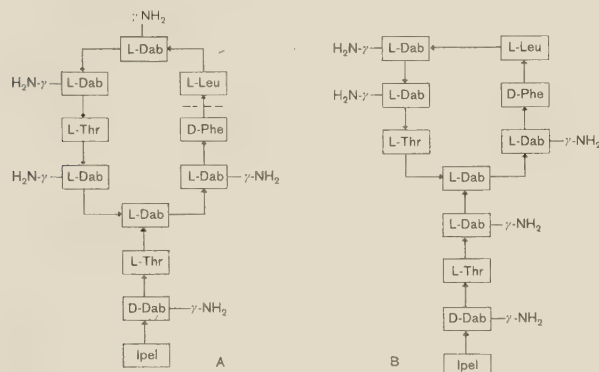


Fig. 1

On Dr. CRAIG's suggestion we are trying to assign the proper constitution to polymyxin B₁ by total synthesis. For this purpose we chose quite arbitrarily two years ago structure A assuming γ -junction between ring and side chain of the completely covered L-Dab residue. In the meantime we performed the synthesis of a decapeptide with the probable structure A in the following manner.

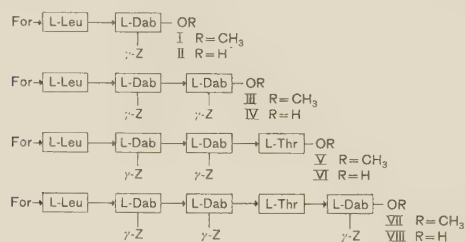


Fig. 2

Preliminary studies showed that the open branched decapeptide XXI (Fig. 4) corresponding to structure A (the ring being opened as indicated by the dotted line in Fig. 1), might be a useful intermediate for its synthesis. XXI was built up from the two protected pentapeptides VIII (Fig. 2) and XX (Fig. 3) exclusively by the carbodiimide procedure⁵ preferably in dimethylformamide as a solvent at low temperature⁶. Ester hydrolysis was achieved

by one mole of aqueous alkali in dioxane or dimethyl sulfoxide.

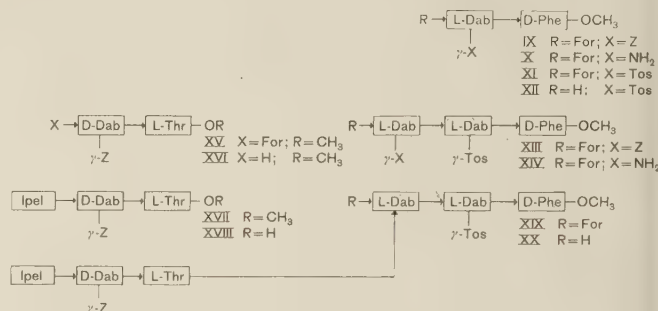


Fig. 3

As protecting groups we needed three residues, which can be removed selectively without interfering with each other. These protecting groups are represented by formyl⁷ benzyloxycarbonyl⁸, and *p*-toluenesulfonyl⁹. The intermediate formyl-L-leucine¹⁰ has been known for a long time and the N γ -benzyloxycarbonyl- α , γ -L-diaminobutyric acid methylester (m.p. 164–166°C) was obtained via the corresponding acid¹¹ in the usual manner. The L-threonine methylester (free base) has been synthesized in crystalline form (m.p. 70–71°C). D-Phenylalanine methylester (free base) was obtained from the hydrochloride¹² by using the chloroform/NH₃ procedure of HILLMANN¹³, whereas N α -formyl-N γ -benzyloxycarbonyl- α , γ -L-diaminobutyric acid (m.p. 98–100°C) was synthesized from the unformylated precursor. (+)-6-Methyloctanoic acid¹⁴ (Ipel) was synthesized according to a new method¹⁵.

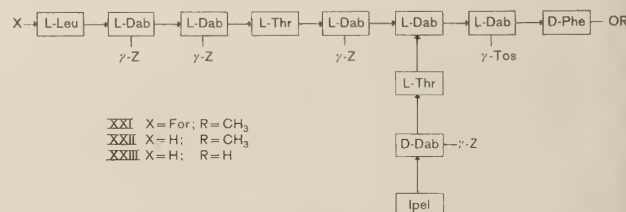


Fig. 4

The other intermediates in Fig. 2, 3, and 4 are new and are characterized in the following Table by their physical constants.

XXI (Fig. 4) after deformylation⁷ led to XXII and subsequent hydrolysis to XXIII. Cyclization by dicyclohexylcarbodiimide⁵ in dioxane/dimethylformamide gave

¹ W. HAUSMANN and L. C. CRAIG, J. Amer. chem. Soc. **76**, 4892 (1954).

² W. HAUSMANN, J. Amer. chem. Soc. **78**, 3663 (1956).

³ G. BISERTE and M. DAUTREVAUX, Bull. Soc. Chim. biol. **39**, 795 (1957).

⁴ Abbreviations for amino acids in this and the following figures have been made in the usual manner according to E. BRAND and J. T. ENSALL, Ann. Rev. Biochem. **16**, 224 (1947); Z = benzyloxycarbonyl, Tos = tosyl and For = formyl, Ipel = isopelargonic acid ((+)-6-methyloctanoic acid); $\rightarrow$ = C to N bond in -CONH-.

⁵ J. C. SHEEHAN and G. P. HESS, J. Amer. chem. Soc. **77**, 1067 (1955).

⁶ G. W. ANDERSON, J. Amer. chem. Soc. **80**, 2902 (1958).

⁷ A. HILLMANN and G. HILLMANN, Z. Naturf. **6b**, 340 (1951). – G. VALEY, Chem. & Ind. **72**, 107 (1953). In the meantime the ability of the formyl-group for peptide synthesis could be demonstrated independently from us by J. C. SHEEHAN and DING DJUNG H. YANG, J. Amer. chem. Soc. **80**, 1154 (1958).

⁸ M. BERGMANN and ZERVAS, Ber. dtsh. chem. Ges. **65**, 1192 (1932).

⁹ V. DU VIGNEAUD and O. K. BEHRENS, J. biol. Chem. **117**, 27 (1936).

¹⁰ E. FISCHER and O. WARBURG, Ber. dtsh. chem. Ges. **38**, 4000 (1901).

¹¹ M. ZAORAL, J. RUDINGER, and F. ŠORM, Chem. Listy **47**, 427 (1953); Chem. Abstr. **49**, 179 (1953).

¹² B. F. ERLANGER, H. SACHS, and E. BRAND, J. Amer. chem. Soc. **76**, 1806 (1954).

¹³ A. HILLMANN, Z. Naturf. **1b**, 682 (1946).

¹⁴ L. CROMBIE and St. H. HARPER, J. Chem. Soc. **1950**, 2685.

¹⁵ To be published.

Table

Product	Crystallized or precipitated from	Melting points uncorrected	$[\alpha]_D^{25} \pm 2^\circ$ (in dimethylformamide)	ϵ at 257 m μ
I	Acetic acid ester	130–131°	– 38,1°	
II	Alcohol/ether	167–168°	– 33,4°	
III	Alcohol	189–190°	– 35,2°	
IV	Methanol	203–205°	– 31,1°	
V	Methanol/ether	227–228°	– 26,1°	
VI	Methanol	211–212°	– 23,6°	
VII	Dimethylformamide/acetic acid ester	205–207°	– 32,8°	
VIII	Methanol/ether	198–200°	– 26,9°	640 (acetic acid)
IX	Alcohol	149–150°	+ 5,8°	
XI	Alcohol	147–148°	+ 4,7°	612 (alcohol)
XIII	Dioxane	194–196°	– 9,2°	818 (alcohol)
XV	Dimethylformamide/acetic acid ester	187–188°	+ 20,3°	
XVI	Acetone	173–174°		
XVII	Acetic acid ester/petroleum ether .	146–147°	+ 29,4°	
XVIII	Acetone	160–161°	+ 35,2°	
XIX	Dimethylformamide/acetone	226–227°	– 4,1°	810 (acetic acid)
XXI	Dimethylformamide/methanol . . .	246–249°	– 15,1°	1507 (acetic acid)

Intermediates X, XII, XIV, and XX have only been obtained as crude materials. All other products were subjected to elementary analysis which agreed with the calculated values. XXI was completely hydrolyzed and assayed (STEIN and MOORE¹⁶) for constituent amino acids, which were found to conform exactly with the proposed structure.

a ninhydrine negative product XXIV (containing all constituent amino acids) from which the free cyclic decapeptide (A) was obtained by removing the protecting groups with sodium in liquid ammonia. Precipitation of the free base from the aqueous salt solution by ammonia at low temperature and redissolving in 0,5 *N* HCl followed by freeze-drying gave the hydrochloride of A, which was used for paper chromatography and microbiological examination.

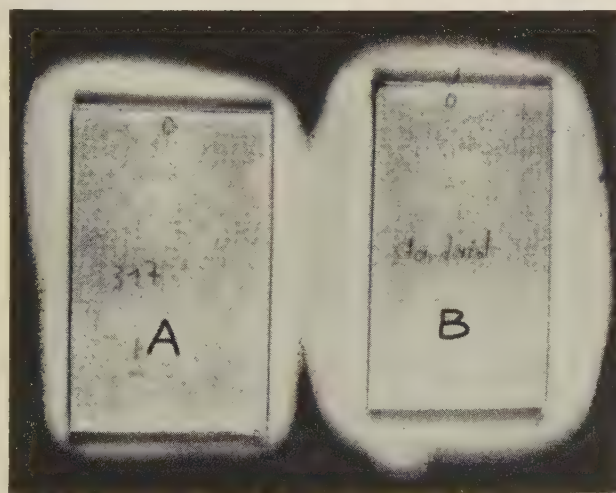


Fig. 5.—A = crude synthetic XXV (A Fig. 1), approx. 300 γ
B = Polymyxin-B-sulfate (Pfizer), approx. 150 γ

Descending paper chromatography carried out as outlined by JONES¹⁷ in the system *n*-butanol/15% aqueous acetic acid, 1:1 (upper phase) for 16 h at 22°C using

Whatman No. 4 paper, gave a running distance of 31,5 cm (solvent front off the leading edge of the paper) for the synthetic A (hydrochloride), whereas commercial Polymyxin-B-sulfate (Pfizer) of natural origin, had a running distance of 33 cm and the open decapeptide (XXIII, without any protecting groups, Fig. 4) of 24 cm.

Microbiological determinations¹⁸ carried out in our Department of Experimental Medicine by Prof. B. FUST and Dr. ERIKA BÖHNI against *Brucella bronchiseptica* with the paper strip directly on the agar, showed a remarkable inhibition zone for the synthetic material, which was used in about twice the concentration of the commercial product (Fig. 5).

The open decapeptide (XXIII, without any protecting groups, Fig. 4) showed less activity against the same microorganism. Further purification of the synthetic material as well as comparison with natural polymyxin B₁ are under way.

Full details will be published in Helvetica Chimica Acta.

K. VÖGLER, P. LANZ, and W. LERGIER

Chemical Research Department of F. Hoffmann-La Roche & Co. Ltd., Basle, July 1, 1959.

Zusammenfassung

Das offenkettige Dekapeptid XXI (Fig. 4) wurde aus den beiden geschützten Pentapeptiden VIII und XX (Fig. 2 und 3) aufgebaut. Die Zyklisierung von XXI führte zu einem mikrobiologisch aktiven Produkt der Formel A (Fig. 1).

¹⁸ *Brucella bronchiseptica* ATCC 4617, Polymyxin assay plate according to FDA, Washington, see also R. G. BENEDICT and F. H. STODOLA, Ann. N. Y. Acad. Sci. 51, 866 (1949).

¹⁶ We are indebted to Prof. M. BRENNER and Dr. R. WEBER from the Institute of Organic Chemistry, University of Basle, for this analysis.

¹⁷ T. S. G. JONES, Ann. N. Y. Acad. Sci. 51, 909 (1949).

Serum para-Phenylenediamine Oxidase
and Xanthine Dehydrogenase Levels
during Liver Carcinogenesis in the Rat

Recently it was reported that the levels of serum para-phenylenediamine (PPD) oxidase and xanthine dehydrogenase (XD) may be temporarily lowered and raised respectively following single intraperitoneal injections of powerful hepatocarcinogens into albino rats¹. We have now studied the changes in the levels of these enzymes during the course of induction of liver tumours by oral administration of 3'-methyl-4-dimethylaminoazobenzene (3'-MeDMAAB) to rats.

Serum samples were obtained by tail-bleeding 10 young adult male albino rats and the enzymes were determined as previously described¹. On the following day (15. 7. 58) the rats were divided into two equal groups, one of which received a daily feed of 100 g of pulverised Diet No. 86 (prepared in pellet form by the North Eastern Agricultural Cooperative Society of Aberdeen) containing 0.06% W/W of 3'-MeDMAAB and made into a paste with tap water (60 ml). The other group received the same regimen but

without the azo dye. Both groups were provided with tap water *ad libitum*. The diets were supplied for 155 days until 8. 12. 58 after which both groups were fed Diet No. 86 until the experiment was terminated on 24. 3. 59 when the last surviving azo-fed rat had to be killed because of a large liver tumour. At intervals, serum PPD oxidase and XD were determined and on several occasions blood haemoglobin and methaemoglobin levels were estimated by the method of VANDENBELT *et al.*².

A statistical analysis of the changes in serum PPD oxidase activity is given in Table I, but data for one of the azo-fed rats has not been included in the analysis. Although this rat responded initially to dye-feeding by a drop in serum PPD oxidase level the decrease was less than that shown by the other 4 rats. Also it behaved abnormally in two other respects. Thus after about 1 month on the diet, the growth of the animal became severely retarded and remained so until it died suddenly on 19. 1. 59 with a moderately large liver tumour. Moreover, the rat developed an anaemia which was more intense than that exhibited by other members of the azo group (see below).

¹ W. J. P. NEISH, *Exper.* **14**, 287 (1958); **15**, 20 (1959); *Naturwissenschaften* **46**, 175 (1959).

² J. M. VANDENBELT, C. PFEIFFER, M. KAISER, and M. SIBERT, *J. Pharmacol. exp. Therap.* **80**, 31 (1944).

Table I
Serum PPD oxidase levels in groups of rats kept on a diet containing the azo dye 3'-MeDMAAB and on a normal diet

Group	1 day before diet commenced		Days after diet commenced			
			21	62	120	154
Azo (n = 4)	mean weight (g)	163 ± 19 ⁺ (139) [†]	182 ± 13 (155)	224 ± 11 (186)	262 ± 16 (194)	269 ± 22 (197)
	PPD range	0.295 – 0.632	0.238 – 0.287	0.161 – 0.222	0.311 – 0.358	0.309 – 0.442
	mean (x _A)	0.502 (0.623) [†]	0.258 (0.488)	0.184 (0.451)	0.332 (0.574)	0.367 (0.572)
	σ _A ² *	0.022346	0.000467	0.000714	0.000378	0.003478
Normal (n = 5)	mean weight (g)	153 ± 12	190 ± 12	228 ± 19	263 ± 23	273 ± 21
	PPD range	0.318 – 0.592	0.311 – 0.456	0.318 – 0.459	0.377 – 0.471	0.330 – 0.612
	mean (x _N)	0.425	0.381	0.361	0.401	0.439
	σ _N ² *	0.011091	0.004211	0.003232	0.001543	0.009020
		t**	4	6.1	3.4	1.5
			(< 5% level)	(> 1% level)	(> 0.1% level)	(1% level)

⁺ Standard deviation

[†] Bracketed values refer to the rat omitted from the analysis.

^{**} t test, $t = \frac{\bar{x}_A - \bar{x}_N}{\sqrt{\frac{\sigma_A^2}{n_A} + \frac{\sigma_N^2}{n_N}}}$

* Best estimate of variance, $\sigma^2 = \frac{\sum (x - \bar{x})^2}{n - 1}$.

Table II

Group	Rat	Days after diet commenced				
		154	189	202	223	251
Azo Diet	1*	13m45s	19m	14m	7m45s	—
	4+	13m15s	20m30s	—	—	—
	5++	not done	12m30s	14m	14m45s	16m30s
Normal Diet	range	8m30s–11m	8m30s–14m30s	9m30s–12m45s	9m30s–12m15s	9m–10m30s
	mean values for	9m45s	11m	11m	11m	10m
		5 rats		4 rats		

* Killed at 223 days; total weight of liver + tumour = 31 g.

+ Killed at 190 days; total weight of liver + tumour = 40 g.

++ Killed at 251 days; total weight of liver + tumour = 44 g.

Table I shows that ingestion of the azo carcinogen led at first to a decline in the serum PPD oxidase levels of the experimental rats. The difference between the mean levels of PPD oxidase activity for experimental and control groups, already pronounced at 21 days, was very marked at 62 days from the start of the feeding experiment. Thereafter, in spite of continued ingestion of the azo dye, serum PPD oxidase levels of the experimental group began to rise and by 154 days there was no significant difference between the mean levels of this enzyme in the experimental and control groups.

When large liver tumours were present in the azo-fed rats, serum PPD oxidase levels remained high (0.4–0.6) so there is no question of permanent suppression or deletion of the enzyme during hepatocarcinogenesis. MILLER and MILLER³ found that the ability of rats to bind azo dye to their liver proteins began to decline after the rats had been fed for about 2 weeks on a diet containing 3'-MeDMAAB. ARCOS and ARCOS⁴ observed that the swelling ability of liver microsomes decreased to a minimum at 4 weeks with the feeding of 3'-MeDMAAB and again increased until normal levels of swelling were attained after 20 to 24 weeks of dye feeding. A diet containing the non-carcinogen 2-methyl-4-dimethylaminoazobenzene failed to influence the swelling of rat liver microsomes after 4 weeks. ROY, MIYA, and CARR⁵ found that the total non-protein sulphhydryl content of the livers of rats fed 4-dimethylaminoazobenzene reached a minimum value after 12 weeks on the diet. After 20–24 weeks, however, the sulphhydryl content had returned to normal levels. In the present experiment we have indications that yet another early effect of the azo carcinogen appears to be overcome as dye-feeding is continued.

Although single intraperitoneal injections of 3'-MeDMAAB as well as of other hepatocarcinogens may produce a temporary increase in serum XD, no significant difference between the mean XD levels of experimental and control groups was found after 21, 62, 120, and 154 days of dye-feeding. It was found however that XD levels of azo-fed rats began to decline within a few weeks after the end of azo dye-feeding. This trend is shown in Table II which also records XD values for control animals. Serum XD levels began to decrease during liver tumour growth and we have noted in another series of rats which had been on a diet of 4-dimethylaminoazobenzene that low serum XD values may be encountered even before liver tumours are palpable. It is of interest that the growth of a transplantable sarcoma Rd/3 in the same rat strain leads to a marked depression in serum XD activity.

Finally, reference is made to the anemic condition of rats fed 3'-MeDMAAB. After 120 days on the diet, hemoglobin levels (as measured by the method of VAN DENBELT *et al.*² and expressed as an optical density difference at 630 m μ) ranged from 0.229 to 0.254 (4 rats) with a mean value of 0.242. The normal group (5 rats) had values in the range 0.276–0.283 with mean = 0.279. Application of the *t* test gave *t* = 6.8 (> 0.1% level) showing that the difference between the means is highly significant. The azo-fed rat excluded from our analyses had a hemoglobin level of 0.195. Essentially the same results were obtained after 154 days on the diet.

The azo carcinogen has a weak methemoglobinogenic action when given orally to rats (a mean value of 1.7% as

compared with 0.2% for the control group at 120 days from the start of dye-feeding) which confirms our previous finding⁶ of this condition following single intraperitoneal injections of 3'-MeDMAAB into rats.

It is noteworthy that all the azo-fed rats developed liver tumours which proved to be cholangiocarcinomata. Attempts to transplant several of these tumours met with no success.

I should like to thank Dr. F. N. GHADIALY for examining histological sections of the tumours, and I am indebted to the University of Sheffield for the James Morrison Fellowship in cancer research.

W. J. P. NEISH

Cancer Research Unit, The University, Sheffield, May 5, 1959.

Zusammenfassung

Werden Ratten mit dem carcinogenen 3'-Methyl-4-dimethylaminoazobenzol gefüttert, so nimmt die Konzentration der p-Phenylendiaminoxidase im Serum anfangs rasch ab, um am Ende der Fütterungsperiode langsam wieder auf den Normalwert anzusteigen.

Eine Verminderung der Xanthindehydrogenase des Serums war nur zu Beginn des Wachstums von Lebertumoren zu beobachten. Mit Azofarbstoffen gefütterte Ratten wurden anämisch.

⁶ W. J. P. NEISH, *Nature* 178, 1350 (1956).

Antibodies in the Course of Resorption of the Ehrlich Cancer Heterotransplant in Rats

Mice tumours heterotransplanted to newborn white rats^{1,2} or to white rat embryos^{3,4} at first grow at a high rate and attain a large size, while in more than two-week old rats they degenerate and are completely resorbed. Resorption of the tumour heterotransplants under these conditions is indicative of lack of acquired tolerance⁴, although tolerance to skin homotransplants can be elaborated in less than 2-week old rats^{5,6}. The mechanism of transplant resorption in 2-week old rats is still obscure.

In the present study, we have followed up the dynamics of antibodies against tumour tissue in sera of rats with resorbing Ehrlich cancer transplant. For this purpose, the agglutination reaction of tumour cells was tested as well as cytotoxic action of sera upon tumour cells in a test tube and *in vivo-in vitro* neutralization.

Methods. The Ehrlich ascitis adenocarcinoma was subcultured to non-inbred strains of white mice. The ascitis fluid was procured from the mice on the 7–12th day after inoculation. The cells were thrice washed with Ringer and 0.25 ml of the suspension of tumour cells containing 160 million cells per 1 ml were injected under the skin of the back of 1–2-day old white rats.

A total of 112 young rats of 12 litters have been inoculated, while 46 normal young rats of 5 litters were used as

¹ I. GEORGIU, *J. Path. Bact.* 29, 171 (1926).

² I. PATTI and A. MOORE, *Cancer Res.* 10, 674 (1950).

³ H. KOPROWSKI, *Proc. R. Soc. [B]* 146, 37 (1956).

⁴ G. J. SVET-MOLDAVSKY, Problems of Oncology (Voprosi onkologii) (in Russian) 4, 552 (1958).

⁵ M. WOODRUFF, *Ann. N.Y. Acad. Sci.* 64, 5 (1957).

⁶ R. BILLINGHAM and L. BRENT, *Proc. [R.] Soc. B.* 146, 78 (1956).

³ E. C. MILLER and J. A. MILLER, *Cancer Res.* 12, 547 (1952).

⁴ J. C. ARCOS and M. ARCOS, *Biochim. biophys. Acta* 28, 9 (1958).

⁵ P.-G. ROY, T. S. MIYA, and C. J. CARR, *Proc. Soc. exp. Biol. Med.* 97, 284 (1958).

Table I

Agglutination of tumour cells by sera of normal rats and of 1-2-day old rats inoculated with Ehrlich tumour

Age of rats	Time after inoculation	Stage of tumour growth	No. of rats	Agglutination titer
1 week	7 days	Intense growth . . .	13	0
2 weeks	14 days	Cessation of growth .	14	0
3 weeks	21 days	Beginning of resorption	7	1: 80
4 weeks	26-27 days	Resorption of half of tumour	11	1:320
4-5 weeks	25-31 days	Complete resorption of tumour	11	1:320
4-5 weeks	28-35 days	1 week after tumour resorption	9	1:160 1:640
7 weeks	44-51 days	4 weeks after tumour resorption	10	1: 80 1:320
1 week	—	Normal rats	13	0
2 weeks	—	Normal rats	18	0
3 weeks	—	Normal rats	8	0
4 weeks and adult	—	Normal rats	10	0

control. The rats were sacrificed 1, 2, and 3 weeks after inoculation, upon a twofold decrease in size of the tumour upon its complete resorption and 1 and 4 weeks after its complete disappearance.

For retransplantation to mice, the tumour growing on young rats was fragmented with scissors, suspended in physiological saline and injected intra peritoneum to 5 mice, 0.2 g tumour tissue to each.

The agglutination of the tumour cells was assayed by the slightly modified HORN⁷ method. To a series of 0.2 or 0.4 ml twice diluted serum 0.2 or 0.4 ml suspension of washed tumour cells were added, containing 1 million cells per ml, the mixture was agitated and incubated for a 1 h at room temperature and 18–24 h at +4°. Agglutination was determined *ad oculo*: +++ was estimated as distinct agglutination at a transparent background. In order to assay the cytotoxic effect *in vitro*^{8,9} to 50 million washed Ehrlich tumour cells were added 0.5 ml of the serum to be tested preliminary kept for 30 min at 56° and 0.5 ml of fresh guinea pig serum (complement) taken on the preceding day. The mixture was agitated and incubated at 37°C. 1, 2, and 4 h thereafter samples of the incubated mixture were stained with eosin diluted 1:1000 in a Thyrode solution, 4 ml eosin being added to 0.05 ml suspension of tumour cells. The percentage of stained cells was then estimated under the microscope. To check *in vivo* the *in vitro* cytotoxic 'neutralizing' effect of the sera, the above mixture of cells, serum and complement incubated for 4 h was administered intraperitoneally to 5 mice, 0.2 ml to each.

Results. On the 5–6th day after inoculation, a tumour was palpated in rats 1 × 2 cm in size. It attained the maximal size, viz. 4 × 2 × 2 cm 11–13 days after inoculation, decreasing in size 1–2 days thereafter and completely disappearing 16–31 days after inoculation.

The maximal increase in size and growth stoppage were approximately simultaneous in all rats of every litter.

⁷ E. HORN, Cancer Res. 16, 595 (1956).

⁸ R. SCHRECK and E. PRESTON, Cancer Res. 17, 102 (1957).

⁹ G. HOSKINS *et al.*, Exp. Cell Res. 6, 11, 297 (1956).

Table II

The cytotoxic effect upon tumour cells of serum of normal rats and of those inoculated with Ehrlich tumour

Age of rats	Stage of tumour growth	% of eosin stained cells 1 h after incubation	% of eosin stained cells 2 h after incubation
1 week	Intense growth	3%	4%
2 weeks	Cessation of growth . . .	81%	90%
3 weeks	Beginning of resorption .	95%	100%
4 weeks	Resorption of one half of tumour	98%	100%
4-5 weeks	Resorption of the whole tumour	90%	92%
4-5 weeks	1 week after resorption .	98%	100%
7 weeks	4 weeks after resorption	90%	90%
4 weeks	Resorption of one half tumour (without complement)	20%	18%
1-3 weeks	Normal rats	3–4%	8–12%
4 weeks	Normal rats	10%	10%
Adults	Normal rats	10%	10%

Tumour resorption proceeded at a different rate in rats of the same litter.

The results obtained in the agglutination reaction of tumour cells by rat sera are summarized in Table I.

The agglutinins appear in the rat serum on the 3rd week after inoculation at a titer of 1:80; 2 weeks after inoculation these antibodies are not yet apparent. In the half tumour resorption group, and in that of complete tumour resorption, the agglutinin titer is 1:320, in that of 1 week after complete tumour resorption 1:160 and 1:640, 1 month after complete resorption 1:80 and 1:320. In most of the sera, the zone phenomenon is apparent: no agglutination by the whole serum and in dilution 1:5 is to be noted.

Sera of young rats with resorbing tumour did not agglutinate mice erythrocytes.

The results of *in vitro* determination of the cytotoxic effect are summarized in Table II. 1–2 h incubation with the serum of rats 2–3 weeks after inoculation and of those with resorbing tumour called forth a distinct cytotoxic effect and increased the percentage of eosin staining cells to 81–100%. Control sera, viz. those of non-inoculated rats of the same age, as well as sera of adult rats and heated experimental sera without complement, did not exhibit any such effect. After a 4-h exposure, no difference between the control and experimental sera could be noted as part of the controls caused an increase in the percentage of stained cells up to 70–90%.

The survival time of mice inoculated with tumour cells incubated with the complement and serum of rats with completely resorbing tumour (a total of 35 mice) averaged 22.7 ± 1.61 days; whereas the survival time of 17 mice inoculated with tumour cells mixed with serum of control rats and complement was 12.7 ± 0.27 days. This difference is statistically significant.

Re-transplantation to mice showed that even the remnants of resorbed tumour can be effectively transplanted to mice.

It will appear from the above evidence that even 2-week old rats can produce antibodies. Sufficient amounts of *in vitro* detectable antibodies appear during the period when no symptoms of decrease in size of the tumours are yet apparent, viz. on the 2nd week of postnatal life. The

agglutinins appear in the serum at a later date, viz. 3 weeks after inoculation when resorption of the tumour is in full sway. Cytotoxins and agglutinins are possibly different antibodies.

The question remains open as to the role of these antibodies in the resorption of the heterotransplant. Re-transplantation of the tumour cells from rats to mice shows that at least part of the tumour cells remain viable in the organism which contains cytotoxins.

Resorption of the mice tumour and appearance of cytotoxins ensues in 2-week old rats while tolerance to homo-transplantation of skin is still demonstrable (BILLINGHAM and BRENT⁴, WOODRUFF⁵).

The capacity of 2- and 3-week old rats to produce antibodies against heterologous tumours throws doubt upon the widely accepted opinion of 'immunological immaturity' of rats of this age.

It is interesting to compare our data and work of HOLLIDAY¹⁰, who has vaccinated 10–11-day old rats with *S. Pullorum* and observed antibody production.

G. J. SVET-MOLDAVSKY and O. S. FRANKFURT

The L. A. Tarasevich State Control Institute of Vaccines and Sera, Moscow, January 27, 1959.

Résumé

Chez les rats jeunes auxquels on a inoculé le cancer ascitique d'Ehrlich 1–2 jours après la naissance, la tumeur atteint une grande dimension et disparaît au bout de 14–31 jours. Chez des rats de 14 jours, le sérum a une action cytotoxique sur les cellules du cancer d'Ehrlich et agglutine celles-ci à partir du 21^e jour.

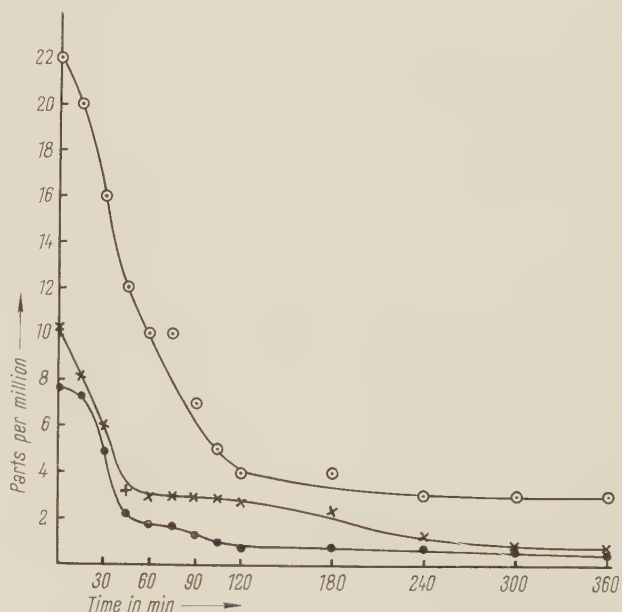
¹⁰ R. HOLLIDAY, Proc. R. Soc. [B] 147, 140 (1957).

Rapid Removal of Phosphorus from Sewage by Activated Sludge

In a study of the growth of plants in the activated sludge tank by setting up what may be termed 'hanging gardens', it was interesting to observe the behaviour of the rice plant which showed an extraordinary vegetative growth, attaining an unusual height and putting forth more than fifty tillers but showing poor formation of grain¹. The rice plant appeared to suffer from deficiency of certain nutrients, notably phosphorus, while it had an excessive or abundant supply of nitrogen. Under the conditions of the experiment, the plants floated on the liquid in the last aeration chamber of the activated sludge tank (the plants being in a suitable box equipped to float, containing a bed of resistant cellulosic material such as *coir* fibre and straw bits) and derived their nutrition from the liquid. The sewage entering the purification tank contained a considerable amount of water-soluble phosphorus, but, as it arrived at the last aeration chamber of the tank after about 4 h of detention with activated sludge, the soluble phosphorus was apparently little or inadequate. It was therefore of interest to study the rate of removal of phosphorus, particularly water-soluble phosphorus, during the purification process. As there is practically no infor-

¹ S. C. PILLAI, Curr. Sci. 10, 85 (1941); Report on *Hanging Gardens* to Dr. G. J. Fowler, Technical Representative of Messrs. Activated Sludge Ltd., London, for India and the East (1941).

mation on these points in the literature on the subject², the evidence we have collected is briefly described in this communication.



Rate of removal of phosphorus from sewage by activated sludge
 —●— water soluble phosphorus (P); —x— total phosphorus (P);
 ○—○ 3 min permanganate value.

Experiments were carried out by adding varying amounts of activated sludge to samples of detritus-free raw sewage (containing generally 10 to 14 p.p.m. of total phosphorus and 6 to 9 p.p.m. of water-soluble phosphorus as determined by the method described by FISKE and SUBBAROW³ and modified by KING⁴; for the determination of water-soluble phosphorus the samples were filtered through filter paper No. 44) and by blowing air through these mixtures (in suitable conical flasks) for varying periods, generally up to 6 h, and by examining the supernatant liquids for their water-soluble and total phosphorus contents, as also for the degree of purification, by customary methods. At the same time, the effect of aeration of samples of sewage only (without the sludge) on their phosphorus contents was also studied.

In one series of experiments, the sewage samples were aerated with 25% sludge for varying periods, from 15 min to 6 h, and the supernatant liquids (collected after stopping the aeration at stated intervals and allowing the contents of the flasks to settle down) were analysed for their phosphorus contents and for the extent of purification as indicated by the 3-min oxygen absorption test (permanganate value) and turbidity. These observations are given in the Figure. In a similar series of experiments, the concentration of sludge was varied while the period of aeration was the same (Table).

² S. H. JENKINS and W. T. LOCKETT, Nature 151, 306 (1943).—C. N. SAWYER, Sewage Wks. J. 16, 925 (1944); J. N. E. Water Works Ass. 61, 925 (1944).—W. RUDOLFS, Sewage Wks. J. 19, 43 (1947).—F. ZEHENDER, Proc. intern. Ass. theor. appl. Limnol. 10, 597 (1948); Water Poll. Abstr. 24, 305 (1951).—E. H. BELCHER, J. Proc. Inst. Sew. Purif. 1951, 348.—C. N. SAWYER, Sewage industr. Wastes 24, 768 (1952).—R. OWEN, Sewage industr. Wastes 25, 548 (1953).—K. WUHRMANN, Sewage industr. Wastes 26, 1 (1954).—T. STONES, J. Proc. Inst. Sew. Purif. 1956, 404.—N. HARKNESS and S. H. JENKINS, J. Proc. Inst. Sew. Purif. 1958, 85.

³ C. H. FISKE and Y. SUBBAROW, J. biol. Chem. 66, 375 (1925).

⁴ E. J. KING, Biochem. J. 26, 292 (1932).

Table.—Influence of Varying Amount of Activated Sludge on the Removal of Phosphorus from Sewage*

Amount of sludge** (percentage) added to sewage and aerated for 6 h	Percentage reduction of turbidity***	Percentage purification based on 3-min permanganate value	Percentage removal of	
			Water soluble Phosphorus (P)	Total Phosphorus (P)
Raw sewage only (control)	4.5	6.9	2.1	1.9
2.5	18.2	22.8	21.7	11.3
5.0	27.3	38.6	26.1	17.7
7.5	40.9	62.8	32.6	40.4
10.0	59.1	78.7	43.5	53.5
15.0	72.8	83.5	82.6	78.1
20.0	77.3	88.3	91.3	90.5
25.0	86.4	88.3	93.2	92.6
30.0	90.9	88.3	94.2	93.6

* The analytical figures for the sewage employed are: turbidity 125; 3-min permanganate value 22 p.p.m.; water-soluble phosphorus (P) 7.7 p.p.m. and total phosphorus (P) 10.3 p.p.m.
** Microscopic examination of the sludge showed the presence of certain protozoa in large numbers, notably the species of *Opercularia* (about 3000 cells per ml) and *Epistylis* (about 800 cells per ml).
*** Turbidity was determined with the aid of a Klett Summerson photoelectric colorimeter, employing violet filter (420 mμ).

The results given in the Figure and the Table show that during the activated sludge process the removal of phosphorus, including the water-soluble phosphorus, from the sewage closely follows the rapid rate of clarification and oxidation of sewage and that the rapidity of removal of the phosphorus, as the purification process, is dependent on the concentration of sludge.

Other observations may be summarised as follows:
When the sludge was previously heated at temperatures above 40°C for 10 min and added to sewage, the removal of phosphorus, as the purification of sewage, was adversely affected. The sludge heated at 50°C for 10 min did not remove the water-soluble phosphorus and did not purify the sewage to any appreciable extent. Treatment of the mixture of sewage and sludge with small amounts of chemicals such as mercuric chloride (4 p.p.m.) also adversely affected both the removal of phosphorus and the purification process. Addition of a mixed culture of bacteria isolated from activated sludge (15 ml bacterial suspension, each ml containing 8000 millions of bacteria, added to 85 ml of raw sewage; the bacteria free from the culture medium were obtained by the technique⁵ using cellophane membrane on nutrient agar) caused only a slight reduction of water-soluble phosphorus but caused an appreciable increase in the total phosphorus, in the permanganate value, and in turbidity, these increases being due to the added suspension of bacteria.

E. G. SRINATH, C. A. SASTRY, and S. C. PILLAI

Department of Biochemistry, Indian Institute of Science, Bangalore (India), April 15, 1959.

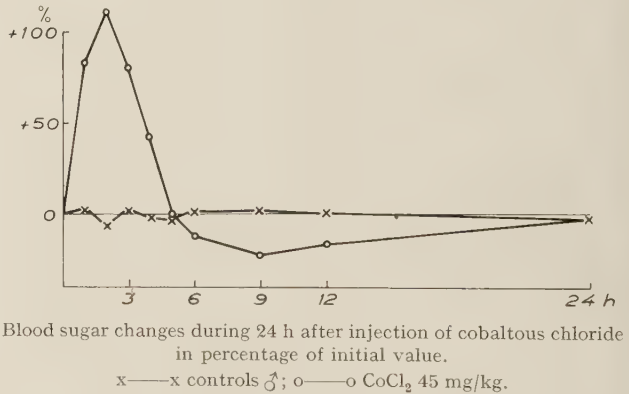
Résumé

La plus grande partie du phosphore contenu dans les eaux d'égouts y compris le phosphore dissous dans l'eau a été rapidement éliminée à l'aide de boue «activée», en l'espace de 6 h. Le liquide recueilli par le procédé de boue «activée» contient très peu de phosphore. Le taux du phosphore éliminé suit de très près la vitesse de purification et d'oxydation des eaux d'égouts. L'activité des bactéries contenues dans la vase ne semble pas expliquer la disparition rapide du phosphore contenu dans les eaux d'égouts.

⁵ T. R. BHASKARAN, M. SREENIVASAYA, and V. SUBRAHMANYAN, Curr. Sci. 3, 484 (1935).

Effect of Cobaltous Chloride on the Blood Sugar Level and the Islet Cells in Rats

Since 1951, when VAN CAMPENHOUT and CORNELIS¹ demonstrated changes in the α-cells of the islets of Langerhans in guinea-pigs after injection of cobaltous chloride, similar observations have been made in other species of animals such as the rabbit and the dog²⁻⁴. Disturbance of the blood sugar level has been reported in a number of cases, the change usually consisting in an increase instead of the decrease that might logically be expected. CREUTZFELDT and SCHMIDT⁵ and FODDEN⁶ have not been able to observe such changes in the blood sugar or islet cells in rats. MUKHERJEE, DE and MUKERJI⁷, on the other hand, obtained hyperglycemia after cobaltous chloride administration (50 mg/kg), but these investigators did not study the islets from the morphological aspect.



¹ E. v. CAMPENHOUT and G. CORNELIS, C. R. Soc. Biol. 145, 933 (1951).
² M. G. GOLDNER, B. W. VOLK, and S. S. LAZARUS, Metabolism 1, 544 (1952).
³ S. S. LAZARUS, M. G. GOLDNER, and B. W. VOLK, Metabolism 2, 513 (1953).
⁴ B. W. VOLK, S. S. LAZARUS, and M. G. GOLDNER, Proc. Soc. exp. Biol. Med., N. Y. 82, 406 (1953).
⁵ W. CREUTZFELDT and W. SCHMIDT, Arch. exp. Path. Pharmac. 222, 487 (1954).
⁶ J. H. FODDEN, Arch. Path. 61, 65 (1956).
⁷ S. K. MUKHERJEE, U. N. DE and B. MUKERJI, Indian J. med. Res. 45, 337 (1957).

Table I
Blood sugar changes during 24 h after injection of cobaltous chloride in percentage of initial value

	Before injection mg/100 g	Blood sugar change in percentage of initial value (h after injection)								
		1	2	3	4	5	6	9	12	24
Controls ♂	92	+2.0	−5.8	+1.8	−1.9	−3.0	+1.5	+1.8	−0.4	−3.3
CoCl ₂ 45 mg/kg ♂ d.f. t	78	+83.4 48 10.86***	+109.5	+78.9 47 8.75***	+42.4	−0.9 47 1.07	−11.9	−21.7 46 3.12** ***	−17.2	−2.8 22 0.056
CoCl ₂ 20 mg/kg ♂ d.f. t	92	+12.9 48 1.613	+5.5	−5.4 47 1.535	−10.9	−14.2 47 3.08***-***	−21.2	−24.6 46 3.66***	−19.7	−18.2 22 1.59
Controls ♀	78	+6.5	+3.8	+1.9	+2.4	+8.6	+1.5	−11.2	−0.9	+1.6
CoCl ₂ 45 mg/kg ♀ d.f. t	73	+131.5 23 4.13***	+103.3	+72.5 24 2.93***-***	+75.8	+6.2 23 0.346	−4.7	+16.3 24 1.046	−3.1	+2.0 11 0.034

* = probable; ** = very probable; *** = significant; d.f. = degrees of freedom; t = Student's t-test.

Material and Methods.—Rats of both sexes were given cobaltous chloride subcutaneously in doses of 20, 35, 40, and 45 mg/kg of body weight and the blood sugar was determined by Hagedorn and Jensen's method once an hour for 6 h and subsequently at longer intervals for 15 days. The animals were sacrificed at different times after the cobaltous chloride injection and the pancreas was fixed in Bouin's fluid and stained by the Gomori method with chromhematoxylin–Ponceau-fuchsin or paraldehydefuchsin–Ponceau-fuchsin. Karyometric examination was carried out by tracing off the nuclear surface of α - and β -cells at a magnification of approximately 2000 and determining the area with a planimeter.

Results.—Following the subcutaneous injection of cobaltous chloride in a dose of 45 mg/kg of body weight a considerable increase in the blood sugar was recorded over a few hours (Table I, Fig.). The finding was the same for both males and females. After 35 or 40 mg/kg, the blood sugar level was also elevated whereas the 20 mg dose did not cause any statistically certain hyperglycemia. About 6–12 h after injection, a hypoglycemic effect was noted in male rats both after 45 and 20 mg/kg of CoCl₂ but not in the females. No disturbances were observed in the blood sugar level at a later stage after the injection (Table II).

The result of the nuclear measurements is shown in Table III; no statistically certain disturbances were demonstrated in the α -cells but there was a slight decrease of the nuclear area in the β -cells 24 h after the injection.

Discussion.—The hyperglycemia observed in this investigation shows a correspondence with the findings obtained by MUKHERJEE *et al.*⁷ in rats. In addition, it was established that doses of 35 and 40 mg/kg of body weight give similar results.

Table II
Blood sugar changes in percentage of starting value over 15 days following injection of cobaltous chloride

	Before injection mg/100 g	Blood sugar change in percentage of starting value (days after injection)		
		2–4	5–8	14–15
Controls ♂	89	+13.5	+7.3	−2.4
CoCl ₂ 45 mg/kg ♂ d.f. t	77	+23.2 55 1.23	+8.5 42 0.214	+3.9 20 0.565
CoCl ₂ 20 mg/kg ♂ d.f. t	87	+7.8 56 0.81	−2.7 43 1.87	−13.0 20 1.044

The possibility that these blood sugar changes might be due to primary disturbances in the cells of Langerhans' islets is contradicted by the karyometric results. It may perhaps be considered remarkable that the appreciable

Table III
Karyometric investigation of α - and β -cells. Nuclear area in μ^2

	α -cells			β -cells			β/α	d.f.	t
	mean	d.f.	t	mean	d.f.	t			
Controls	25.5			28.8			1.13		
1½ h after CoCl ₂ inject.	25.1	8	0.552	28.7	8	0.182	1.14	8	0.303
3 h after CoCl ₂ injection	25.6	7	0.106	28.1	7	1.051	1.10	7	0.772
6 h after CoCl ₂ injection	25.8	7	0.318	28.7	7	0.164	1.12	7	0.21
12 h after CoCl ₂ injection	25.0	8	0.521	26.9	8	1.96	1.08	8	1.296
24 h after CoCl ₂ injection	25.1	8	0.525	27.2	8	3.34**	1.09	8	1.093

blood sugar disturbances did not produce any effect on the α -cells and caused only a slight reaction in the β -cells, from what can be judged from the karyometric results.

There would thus seem to be some reason for assuming that the hyperglycemia has some extra-insular source. Disappearance of the blood sugar elevation has also been observed after cobaltous chloride administration in experimental animals with excised adrenal glands^{8,9}, or after preceding treatment with dihydroergotamine¹⁰, and it therefore seems probable that in rats also the effect produced by cobaltous chloride might be in some way connected with the adrenal glands.

Supported by grants from the Swedish Medical Research Council.

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Department of Pathology, University of Uppsala (Sweden), April 3, 1959.

Résumé

L'effet de l'administration de chlorure de cobalt chez le rat normal est une hyperglycémie prononcée dans l'espace de quelques heures après l'injection. Il n'est pas possible de déceler des changements dans les cellules α des îlots de Langerhans. Les noyaux des cellules β ne montrent qu'une diminution faible après 24 h de l'injection.

⁸ C. v. HOLT and L. v. HOLT, Z. Naturforsch. 9b, 319 (1954).

⁹ C. FRANCK, M. LAMARCHE, and R. KOCAREV, C. R. Acad. Sci. 245, 1165 (1957).

¹⁰ S. ELLIS, H. L. ANDERSON, and M. C. COLLINS, Proc. Soc. exp. Biol. Med., N.Y. 84, 383 (1953).

Dehydrogenase Activity of *Borrelia recurrentis*

So far, little is known about the metabolism of pathogenic spirochetes, since their mass cultivation *in vitro* in a defined medium has only been partially successful^{1,2}. Recently, BUCCA *et al.*³ and BARBAN⁴ reported about certain metabolic observations; however, they used in their studies the non-pathogenic Reiter strain of *Treponema* for testing.

In the present work, the dehydrogenase activity of a known pathogen, *Borrelia recurrentis*, was investigated in a fairly well defined medium, using Tetrazolium reduction as an indicator.

Suspensions of washed *Borrelia recurrentis*⁵, containing 10^5 – 10^7 spirochetes per mm³, were prepared as follows: Mice infected with the organisms were exsanguinated and the blood placed in an anticoagulant fluid (1 p sodium citrate (3%), 1 p saline (0.85%) and 2 p tryptose-phosphate broth (Difco)). The spirochetes were sedimented by spinning and resuspended in fresh fluid (2 p saline and 2 p tryptose-phosphate broth). Several washings were then made, to remove all extraneous materials. The washed spirochetes remained viable for at least 12–15 h in the wash fluid without added nutrients.

The various dehydrogenase systems to be tested were made up in test tubes containing (1) 0.5 ml of the spirochete suspension; (2) 0.1 ml of a 1% solution of one of a number of substrates (glucose, pyruvate, succinate, fumarate, lactate, formate, glutamate, and asparagine); (3) 0.1 ml of a 0.05% solution of either cysteine, cystine, oxidized or reduced glutathione, or ascorbate; (4) 1 ml of freshly prepared 1:10 dilution made from a 1% stock solution of 2, 3, 5 triphenyltetrazolium chloride in phosphate-saline buffer, pH 6.85 (TTC); and (5) saline (0.85%) to bring the total volume up to 2 ml. In the controls, either the borrelia suspension or one of the substrate components was replaced by buffered saline. All tubes were layered with petrolatum and incubated in a water bath for periods up to 6 h at 30°C. Higher incubation temperatures tended to have detrimental effects on the spirochetes. It was also observed that a pH of 6.85 eliminated the troublesome auto-reduction of the TTC, which frequently occurred at a pH of 7, or above.

Results.—On preliminary examination, the dehydrogenase systems concerned with oxidation of all substrates were found either equivocal or negative. After ruling out the possibility that inadequacy of pH was responsible for these results, a deficiency of appropriate coenzymes was considered. It has already been noted that addition of fresh serum somewhat enhanced activity of the borrelias. However, addition of DPN (0.1%) only slightly improved the results. Apparently, during preparation of the microbial suspension, sufficient coenzymes were carried over so that additional DPN had but little influence.

Since it is known that SH-compounds have the ability to activate several dehydrogenase systems, compounds such as cysteine, cystine, reduced and oxidized glutathione were added to the various test systems. The reaction which was previously weak and unduly protracted (requiring overnight incubation) became accelerated in presence of the reduced agents (less than 6 h) but remained inactive with the oxidized form of compounds. Under the experimental conditions employed, the most active enzyme was one attacking the formate; but following it, the other substrates were also oxidized after variable lapse of time.

In the controls, in the presence of heat-killed organisms, no reduction occurred. Similarly, omission of either of the substrates or of the borrelias failed to reduce the dye.

It remained to be seen whether the SH-compounds acted specifically or not. Using ascorbate as an alternate reducing agent, the very same results were obtained as with SH-compounds. Hence there can be little doubt that the catalytic effect is not specific and is not limited to SH-compounds alone.

The activation phenomenon may be explained by assuming that the enzymes became inactivated in the course of preparation (due to oxidation or perhaps due to trace metal poisoning), and reactivated by adequate reduction or neutralization of the metal poisons.

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Department of Microbiology and Public Health, The Chicago Medical School, Chicago 12 (Ill.), May 19, 1959.

Zusammenfassung

Es wurde gezeigt, dass *Bor. recurrentis* unter üblichen Bedingungen nur eine schwache Fähigkeit besitzt, verschiedene Substrate zu dehydrogenieren. In Gegenwart von reduzierenden Agentien (wie SH-Verbindungen oder Ascorbinsäure) wird diese Aktivität hingegen bedeutend verstärkt.

¹ Q. M. GEIMAN, Ann. Rev. Microbiol. 6, 299 (1952).

² W. SCHMEROLD, Zbl. Bakt. I. O. 166, 274, 282, 291 (1956).

³ M. A. BUCCA, J. D. THAYER, H. B. ROBERTS, and G. TAGER, J. Venerol. Dis. Inform. 32, 6 (1951).

⁴ S. Barban, J. Bact. 68, 493 (1954); 71, 274 (1956).

⁵ Received from Dr. N. ERCOLI, Armour Research Laboratories, Kankakee, Ill.

Influence of Heparin and Serotonin on Basophil Leucocytes of Rabbit Blood¹

Both the tissue mast cell and the basophil leucocyte hold metachromatic mucopolysaccharide-containing granules. JORPES *et al.*² claimed that the mast cell granules contain heparin. TAUBERT³ in 1951 extracted a substance with heparin-like activity from blood basophils of a patient suffering from chronic myeloid leukemia. PIETTE and PIETTE⁴, using histochemical methods, suggested the presence of different stages of heparin synthesis in human and rabbit basophils.

Serotonin (5-hydroxytryptamine) has also been demonstrated in tissue mast cells of rats and mice, but not in man or rabbit⁵. No report concerning the presence of serotonin in the basophil leucocyte has been published.

In the present investigation, the influence of systemic administration of heparin and its antagonist protamine, as well as of serotonin and its releaser reserpine, on rabbit blood basophils has been studied.

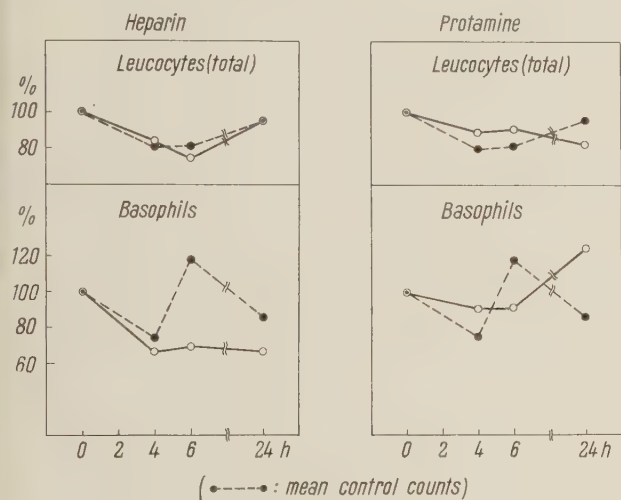


Fig. 1.—Effect of intravenous injection of heparin (1.5 mg/kg) and protamine (0.8 mg/kg) on basophil and all white cell counts of rabbit blood (mean for 10 rabbits).

Methods.—Male white rabbits of 2000–2500 g body weight were caged separately and not used until they had become accustomed to handling. Heparin (Leo, Copenhagen): 1.5 mg/kg, protamine (Roche, Basel): 0.8 mg/kg, serotonin (Nutritional Biochemical Corp., Ohio): 15 mg/kg, and reserpine: 5 mg/kg, were injected slowly at about 9 a.m. into ear veins of groups of 10, 10, 5, and 5 rabbits respectively. Blood samples were obtained immediately before as well as 2, 4, 6, and 24 h after injection. In another group of 10 rabbits (injected in the same way with serotonin), the counts were performed every morning at 9 a.m. for 4 successive days before and 3 days after a single intravenous serotonin injection.

The blood was diluted 1:10 with a modified⁶ toluidine blue diluting fluid⁷ for direct counting of basophils and all white blood cells in Fuchs-Rosenthal counting chambers.

Control counts were performed similarly, on the previous day in relation to injections of the solvent instead of the test-agent in the same animals.

Results.—Heparin injection was followed by a significant fall ($P < 0.02$) in the number of basophils within 6 h, while protamine sulphate did not produce any significant change (Fig. 1).

6 h after administration of serotonin, an increase in the number of circulating basophils was observed, though statistically non-significant. This basophilia, however, seems to be part of a generalized leucocytosis (Fig. 2). In the other group of 10 rabbits, 24 h after serotonin injection, the basophil count proved to be significantly higher than that of the mean control count ($0.02 < P < 0.05$), the increase in the total leucocyte count was non-significant. The basophil and the total white blood cell count then returned to the control level within 3 days

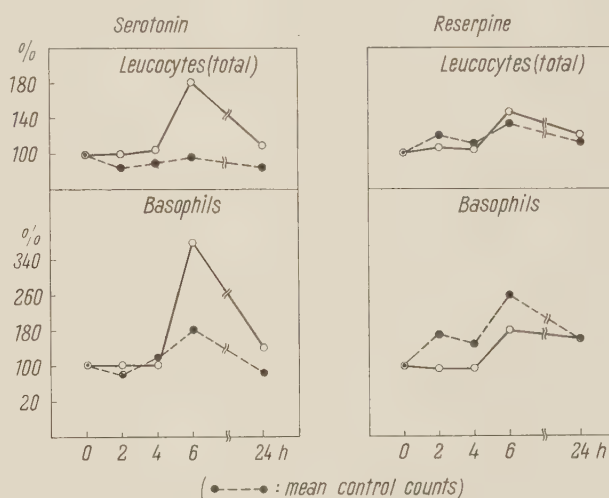


Fig. 2.—Effect of intravenous injection of serotonin (15 mg/kg) and reserpine (5 mg/kg) on basophil and total white cell counts in rabbit blood (mean for 5 rabbits).

(Fig. 3). Reserpine, on the other hand, did not induce any change in the basophil or the total leucocyte count (Fig. 2).

Discussion.—Following intravenous administration of heparin in man, ANGELI *et al.*⁸ reported an increase in the number of circulating basophils, reaching a peak in 2 h, coincident with a rapid decrease in the anti-coagulant activity of the plasma. They attributed this effect to a specific regulatory action where the basophils 'fix' the circulating heparin. However, EIBER and DANISHEFSKY⁹ reported that intravenously injected radioactive heparin is cleared from the blood of man within 3–7 h. Moreover, BOSEILA and ASBOE-HANSEN¹⁰ did not find any increase

¹ This work was supported by grants from the Danish Rheumatism Association, and Eli Lilly and Co., Indianapolis, Indiana, U.S.A., to Dr. ASBOE-HANSEN.

² E. JORPES, H. HOLMGREN, and O. WILANDER, *Z. mikr. anat. Forsch.* 42, 279 (1937).

³ M. VON TAUBERT, *Z. ges. inn. Med.* 6, 758 (1951).

⁴ M. PIETTE and C. PIETTE, *C. R. Acad. Sci., Paris* 242, 2776 (1956); 244, 499 (1957).

⁵ J. R. PARRATT and G. B. WEST, *J. Physiol.* 137, 169 (1957).

⁶ A.-W. A. BOSEILA, *Proc. Soc. exp. Biol. Med., N.Y.* 98, 184 (1958).

⁷ J. E. MOORE and G. W. JAMES, *Proc. Soc. exp. Biol. Med., N.Y.* 82, 601 (1953).

⁸ G. ANGELI, G. TEDESCHI, and F. CAVAZZUTI, *Minerva med.* 46, 752 (1955).

⁹ H. B. EIBER and I. DANISHEFSKY, *A. M. A. Arch. int. Med.* 102, 189 (1958).

¹⁰ A.-W. A. BOSEILA and G. ASBOE-HANSEN, *Proc. Soc. exp. Biol. Med., N.Y.*, 101, 198 (1959).

in the number of metachromatically granulated leucocytes following incubation of rabbit blood *in vitro* with heparin. So, it seems more likely that the observed changes in the number of circulating basophils (increase, or decrease as in the present experiment) reflect a generalized systemic reaction to heparin administration.

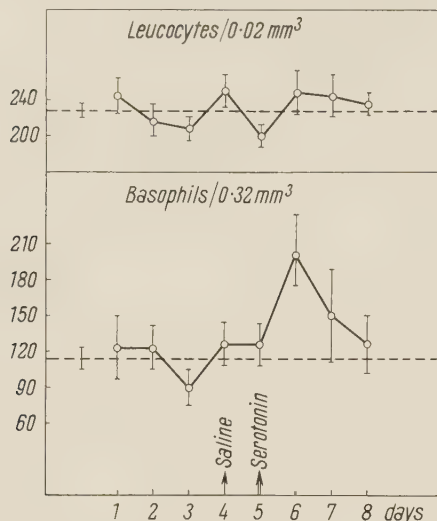


Fig. 3.—Effect of intravenous injection of serotonin (15 mg/kg) on morning counts of basophil and all white cells in rabbit blood (mean for 10 rabbits; standard error of the means indicated by vertical lines; --- indicates the mean count of 4 control days).

Serotonin injection caused an increase in the number of circulating basophils in rabbit blood, significant only after 24 h, but it did not produce a specific, quick, and highly significant basophilia comparable to that occurring within 4 h as a response to histamine administration in the rabbit¹¹. Whereas in the rabbit, histamine is a highly potent edema-producing factor, acting by an increase of capillary permeability, serotonin produces but a negligible increase in tissue water in this species¹². This difference may explain the non-conformity between the actions of the two agents on the blood basophils in the rabbit.

Under the conditions of the present experiment, neither protamine nor reserpine seems to induce a measurable change in the number of circulating basophils of rabbit.

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Connective Tissue Research Laboratory, Institute of Medical Anatomy, Copenhagen (Denmark), May 8, 1959.

Résumé

Chez le lapin, l'injection intraveineuse d'héparine a été suivie d'une diminution significative du nombre des basophiles du sang au cours de 6 h. La sérotonine a produit pendant ce temps une augmentation du nombre total des leucocytes, et au bout de 24 h, une augmentation spécifique du nombre des basophiles.

Les basophiles n'ont pas changé de nombre après l'injection de protamine ou de réserpine.

¹¹ A.-W. A. BOSEILA, *Acta allergol.*, in press (1959).

¹² D. WILHELM, in *5-Hydroxytryptamine* (Pergamon Press, London 1958), Discussion p. 178.

¹³ Author on leave from the Histology Department, Faculty of Medicine, Cairo University (Egypt).

Studies on a Possible Method of Separation of γ -Casein by High-Speed Centrifugation and its Identification by Agar Electrophoresis

By high-speed centrifugation, various portions of casein separate out, according to their contents in the milk and the applied centrifugal force. HEYNDRIKX and VLEESCHAUWER^{1,2} subjected milk, and its sera obtained by centrifugation, to moving boundary electrophoresis. From the results of investigation on centrifuged and non-centrifuged milk, the authors concluded that three caseins occur in milk, and identified the α - and β -components. They made no reference to γ -casein, but mentioned that the component which settled down faster and showed a decrease from 9.3 to 3.6% in the milk serum was also of a casein character.

The present report provides additional information as to the nature of the casein fraction that settles down on high-speed centrifugation at low temperature. Using a suitable concentration of the sediment, it has been possible to demonstrate by agar electrophoresis³ the presence of a distinct band of γ -casein, and also three bands of casein fractions, in a mixed solution of the sediment and of the caseins precipitated from the clear serum.

Method.—Milk was dialysed in the cold in cellophane bags for 72 h against veronal buffer at pH 8.6 and ionic strength of 0.1. An aliquot of 20 ml of dialysed milk was centrifuged at 10,000 r.p.m. in the cold (2°C) for 30 min. This separated the fat out as a solid mass at the top leaving a clear serum, and a compact sediment at the bottom.

Treatment of the sediment: After removal of the layer of fat, the clear serum was decanted off completely. The sediment was washed twice with a solution of *N*/50 acetate buffer of pH 6.0 and then with cold ether. The sediment was finally dissolved in 1.0 ml of KCl-borate buffer solution of pH 10.0 (Solution 1).

Treatment of the serum: The casein fractions in the clear serum were precipitated at pH 4.6 with dilute acetic acid and centrifuged. The precipitate from 5.0 ml of clear serum was washed with cold ether and dissolved in 2.0 ml of KCl-borate buffer solution (Solution 2).

Solution 3 is a mixture of equal volumes of solution 1 and Solution 2 and, therefore, contained all the casein fractions present in milk. The concentration of the casein fraction that separated as a sediment on centrifugation was at a higher level in solution 3 as compared to that present in the original milk.

The solutions were subjected to agar electrophoresis for 6 h at 250 volts and a current strength of 8 mA. The electrophoretic diagrams are shown below in the following figures.

Discussion.—Figure 1 shows a distinct band due to the casein fraction present in the sediment. In Figure 2, the same band is seen against two distinct bands due to the casein components present in the clear serum. The single band at the top having the lowest mobility is presumably due to γ -casein, while the two lower bands probably correspond to β - and α -caseins in the order of increasing mobility.

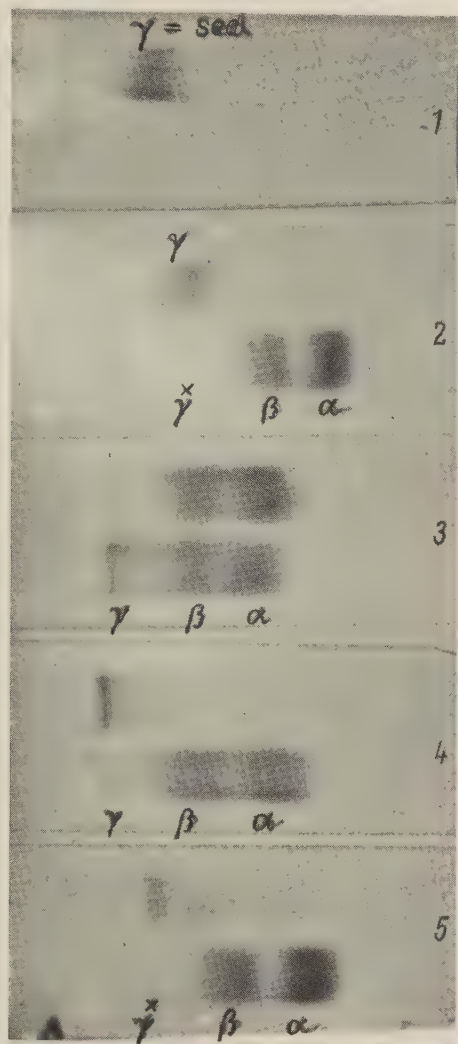
Figure 3 depicts the three bands due to α -, β -, and γ -caseins in the mixed solution, the band of γ -casein being

¹ G. V. HEYNDRIKX and A. DE VLEESCHAUWER, *Exper.* 8, 317 (1952).

² G. V. HEYNDRIKX and A. DE VLEESCHAUWER, *Biochim. biophys. Acta* 6, 487 (1951).

³ K. V. GIRI, *Naturwissenschaften* 43, 232 (1956).

demonstrable as its concentration in the mixed solution was ten times that in original milk.



In Figure 4 the least mobile fraction in the mixed solution exactly corresponds to the position occupied by the single band due to sediment solution (Solution 1) at the top.

From a study of the electrophoretic diagrams, it is evident that the position of γ -casein singly and also in the mixture is quite distinct and characteristic. That a portion of β -casein also settles down in the sediment at higher speed of centrifugation became evident when the solution of such sediment was subjected to electrophoresis for longer periods using 0.5% agar (Fig. 5).

It is, therefore, concluded that the sediment that settles down on high-speed centrifugation comprises mostly of γ -casein.

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Department of Biochemistry, Indian Institute of Science, Bangalore, and Bangalore Medical College, March 16, 1959.

Résumé

Le présent article traite de la possibilité d'obtenir une méthode de séparation du γ -caséine par centrifugation à grande vitesse et à basse température et son identification par l'électrophorèse avec la gelée.

* Deceased.

Der Einbau von radioaktiv markiertem Schwefel (^{35}S) in den Knorpel normaler und Vitamin-C-frei ernährter Meerschweinchen

Die Untersuchungen von DZIEWIATKOWSKI *et al.*^{1,2} und von BOSTRÖM *et al.*³⁻⁵ an Ratten haben ergeben, dass nach intraperitonealer Injektion von anorganisch gebundenem Schwefel (Sulfat) dieser zum Aufbau von Knorpelgewebe, insbesondere von Chondroitinschwefelsäure, verwertet wird. Das Mucopolysaccharid Chondroitinschwefelsäure ist der wesentlichste Bestandteil des hyalinen Knorpels. Es zeigte sich, dass 24 h nach der Injektion das Maximum der Schwefelaufnahme erreicht ist; von diesem Zeitpunkt an nimmt die Radioaktivität des Knorpelgewebes allmählich wieder ab und geht nach rund 16 Tagen auf die Hälfte des Maximalwertes zurück (BOSTRÖM³). REDDI und NÖRSTROM⁶ konnten ferner nachweisen, dass die Verwertung von Schwefel bei Vitamin-C-frei ernährten Meerschweinchen wesentlich schlechter ist als bei Normaltieren. So fand sich in den Kostalknorpeln von Vitamin-C-Mangeltieren, 48 h nach der Injektion markierter Sulfatlösung, nur rund ein Drittel der Menge radioaktiver Chondroitinschwefelsäure, wie sie bei gesunden, normal ernährten Tieren im Knorpel ermittelt werden konnte.

Im Rahmen eigener Untersuchungen über den Stoffwechsel des Knorpelgewebes führten wir unter anderem zwei Versuchsreihen durch, deren Ergebnisse den Befund von REDDI und NÖRSTROM vollauf bestätigen konnten. Es sei dies hier kurz mitgeteilt.

Wir benutzten zu den Versuchen ausgewachsene, weibliche Meerschweinchen. Die Vitamin-C-Mangeltiere erhielten als Nahrung Grundfutter (Biskuits aus Haferflocken, Kleie, Magermilchpulver, Lebertran u. a.), sterilisiertes Heu und Wasser, während die Normaltiere Grundfutter, normales Heu und Runkelrüben bekamen; ausserdem wurde jedem Normaltier täglich 10 mg Ascorbinsäure injiziert. Zwei Tage vor der Sektion, die auf den 17. bzw. 18. Skorbuttag fiel, wurde allen Tieren intraperitoneal 1,5 ml trägerfreie Natriumsulfatlösung (^{35}S) injiziert, $1,5 \times 10^6$ cpm pro Tier. Die 12 seziierten Normaltiere hatten ein Durchschnittsgewicht von 280–300 g, während die 36 überlebenden Skorbuttiere am Tage der Sektion nur noch 180–200 g schwer waren. Die Kostalknorpel von je zwei Tieren wurden gemeinsam verarbeitet. Nach Aufschliessen des getrockneten Knorpelmateriale mit der Methode nach BAILEY⁷ und Zugabe von 0,25 ml normaler Natriumsulfat-Lösung als Trägersubstanz wurde der Gesamtschwefel durch Bariumchlorid gefällt und die Radioaktivität des Sulfatniederschlages bestimmt.

Aus der Tabelle ist zu ersehen, dass von den Normaltieren rund doppelt soviel Schwefel in die Kostalknorpel eingelagert wurde wie von den Skorbuttieren. Dieser Befund bestätigt die Versuchsergebnisse von REDDI und NÖRSTROM, die bei Normaltieren dreimal mehr radioaktiven Schwefel feststellten als bei Vitamin-C-Mangeltieren. Der graduelle Unterschied zu unseren Zahlen beruht wohl darauf, dass REDDI und NÖRSTROM die injizierte Menge aktiven Schwefels auf je 100 g Körpergewicht pro Tier berechneten, während wir allen Tieren gleichviel Schwefel injizierten. Die wesentlich schwereren

¹ D. D. DZIEWIATKOWSKI, R. E. BENESCH und R. BENESCH, J. biol. Chem. 178, 931 (1949).

² D. D. DZIEWIATKOWSKI, J. biol. Chem. 189, 187 (1951).

³ H. BOSTRÖM, J. biol. Chem. 196, 477 (1952).

⁴ H. BOSTRÖM und S. ÅQVIST, Acta chem. scand. 6, 1557 (1952).

⁵ H. BOSTRÖM und E. JORPES, Exper. 10, 392 (1954).

⁶ K. K. REDDI und A. NÖRSTROM, Nature 173, 1232 (1954).

⁷ K. BAILEY, Biochem. J. 31, 1396 (1937).

Anzahl	Mittleres Trockengewicht der Kostalknorpel von je zwei Tieren	cpm/g Trockengewicht Mittelwert	Statistische Sicherung
12 Normaltiere, Gewicht 280–300 g. 36 Skorbuttiere, Gewicht 180–200 g.	0,45 g 0,38 g	930 ± 148 cpm <i>n</i> = 6 475 ± 34 cpm <i>n</i> = 18	Unterschied stark gesichert (<i>t</i> -Verteilung)

Normaltiere erhielten infolgedessen in unseren Versuchen, bezogen auf ihr Körpergewicht, rund ein Drittel weniger Schwefel als die leichteren Skorbuttiere. Dieser Umstand macht es erklärlich, dass die Differenz des aufgenommenen Schwefels zwischen Normal- und Skorbuttieren in unseren Versuchsreihen um rund ein Drittel kleiner war als jene der Tiere von REDDI und NÖRSTROM.

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Rheumaklinik und Institut für Physikalische Therapie der Universität Zürich und Robapharm AG., Basel, 27. April 1959.

Summary

The utilization of sulphate labelled with S³⁵ to the synthesis of chondroitin sulphate of the cartilage of normal and scorbutic guinea pigs has been examined. The average incorporation of S³⁵ in the costal cartilage in the deficient animal was about one half that of the normal.

Changes in Cerebral Blood Supply Caused by Changes in the Pressure Drop along Arteries to the Brain of the Cat

The resistance of a vascular bed is usually estimated from simultaneous records of the blood flow and the arteriovenous pressure drop. Since the major part of this pressure decrement is usually believed to occur at the level of the terminal arteries and arterioles¹, the upstream pressure is regarded as being equal to the systemic blood pressure which is recorded in a large artery. However, when arteries are long and narrow, their resistances cannot be neglected. In such cases, there may also be a fall in pressure in the arteries themselves and this may vary in relation to changes in arterial blood flow and to changes in arterial calibre. As a consequence, when a number of vascular beds are coupled in parallel at the end of a narrow artery, a strong vasodilatation in one of them may cause a redistribution of the flow of blood at the expense of the others.

The object of the present investigation was to determine whether or not the resistance of the arteries to the cat's brain is large enough to influence the cerebral blood flow (SCHMIDT and HENDRIX²). The arterial anatomy of the head of the cat differs markedly from that of the human, for in the cat the internal carotids are usually small or absent whereas the external carotids have anastomotic branches to the circle of Willis through a *rete mirabile*³. The basilar artery is usually well developed. The relative proportion of carotid and vertebral arterial

pressures determines the position of the 'dead point' of flow in the arteries below the base of the brain⁴. Under normal conditions, this point is situated in the rostral end of the basilar artery. We have tried to solve our problem by comparing cortical blood flow and pressure in the anterior part of the basilar artery during various experimental conditions. Since this pressure was sometimes found to be much lower than the systemic blood pressure, our next problem was to determine whether the pressure decreased all along the cranial arteries, or whether the fall was confined to the abovementioned *rete*. The physiological effect of the pressure drop in the cranial arteries was also studied by recording the electrocorticogram.

Methods.—All the cats were anaesthetized with 'Nembutal' (40 mg/kg intraperitoneally). A few of them were, in addition, immobilized with 'Flaxedil' and artificially ventilated. Blood pressures were recorded by strain gauge manometers which showed very little change in volume during large changes in pressure. The bones of the base of the skull were removed, and the basilar artery was exposed, ligated and cannulated with a fine nylon tube or glass pipette connected to the transducer with a wide-bore polyethylene catheter. The systemic blood pressure was measured through a catheter in the femoral or iliac artery. Cortical blood flow was determined by the method of INGVAR and SÖDERBERG⁵ in which the outflow from the cannulated superior sagittal sinus is recorded with an electronic drop counter. The bones of the skull were removed as far as was necessary to interrupt all important anastomoses to the diploic veins. The animal was completely heparinized and the sinus cannulated. The dura was covered with cellulose sponge and the bony defect repaired with dental acrylate cement, molded to form a watertight seal around the sinus cannula. The free end of the cannula was kept in a fixed position relative to the sinus. The blood was returned to the animal by intravenous infusion at a rate equal to the outflow. The electrocorticogram (EEG) was obtained with silver ball electrodes implanted epidurally and an Offner electroencephalograph, type A.

Results.—In animals judged to be in good condition, the basilar artery pressure was initially 70 to 90% of the systemic level. The pulse pressure recorded was usually very small even when the pressure was measured with a transducer that had a volume deflection as low as 0.1 mm³/100 mm Hg⁶.

We have confirmed the observation of SCHMIDT and HENDRIX that extracranial vasoconstriction, e.g. elicited by weak sympathetic stimulation, favoured the flow of blood to the brain. This effect is due to a reduction in the pressure drop from the systemic circulation to the cerebral arteries, as a consequence of reduced blood flow through those extra-cranial tissues that are supplied by the external carotids. Conversely this pressure drop was exaggerated during a state of extracranial vasodilatation caused, for example, by section of the sympathetic nerves.

¹ E. M. LANDIS, *Heart* 15, 209 (1929–1931).

² C. F. SCHMIDT and J. P. HENDRIX, *Res. Publ. Ass. nerv. ment. Dis.* 18, 229 (1937).—C. F. SCHMIDT, *The Cerebral Circulation in Health and Disease* (Thomas, Springfield 1950).—U. SÖDERBERG and N. WECKMAN, *Acta radiol.* 50, 317 (1958).

³ P. M. DANIEL, J. D. K. DAWES, and M. M. L. PRICHARD, *Philos. Trans. [B]* 237, 173 (1953).

⁴ D. A. McDONALD and J. M. POTTER, *J. Physiol.* 114, 356 (1951).

⁵ D. H. INGVAR and U. SÖDERBERG, *Acta physiol. scand.* 42, 130 (1958).

⁶ Statham transducer P23 g.

Strong sympathetic stimulation was required to produce intracranial vasoconstriction. This diminished cortical blood flow⁷, but, as a rule, also reduced basilar pressure (Fig. 1 *a* and *b*). We have concluded from our records and from direct inspection of the blood vessels that this reduction in cerebral blood flow is mainly due to constriction of arteries.

On the other hand, vasodilatation of small cerebral vessels, elicited by acetylcholine (Fig. 1 *c*), ether and some other substances, was sometimes more pronounced than one would expect from comparing records of systemic pressure and of cortical blood flow, because there was generally an increase in the pressure gradient from the carotid to the basilar artery.

The basilar pressure was not a linear function of the systemic pressure when the latter was altered artificially. There seems to be increased arterial tone in response to very high pressures. In addition, very low pressures favoured the constriction that could be induced by sympathetic stimulation. However, the effects are difficult to evaluate because of the changes in blood flow that follow altered pressure.

Noradrenaline elicited the same effects as sympathetic stimulation but arterial constriction could only be induced by fairly unphysiological doses (Fig. 1 *d*, *e*, and *f*). There was a synergism between sympathetic activity and noradrenaline. The arterial constriction elicited by these procedures was also favoured by low systemic blood pressure and by mechanical stimulation of the arteries, e.g. during the dissection at the beginning of the experiment. Adrenaline had an effect similar to that of noradrenaline but weaker, and was also found to dilate small cerebral vessels.

The arterial spasm seen after strong sympathetic stimulation could lead to complete occlusion of the vessels. This occurred both at the carotid level and inside the skull, and was demonstrated by clamping the carotid artery. In those cases where the carotid was already occluded on one side, a clamp on the same side had no effect on the basilar pressure or on the cerebral blood flow. When arteries inside the skull were strongly con-

stricted, a clamp on the carotid caused a further reduction of the basilar pressure. However, this experiment cannot be done in all animals since there is often a considerable asymmetry in the vascular supply of the cat's brain.

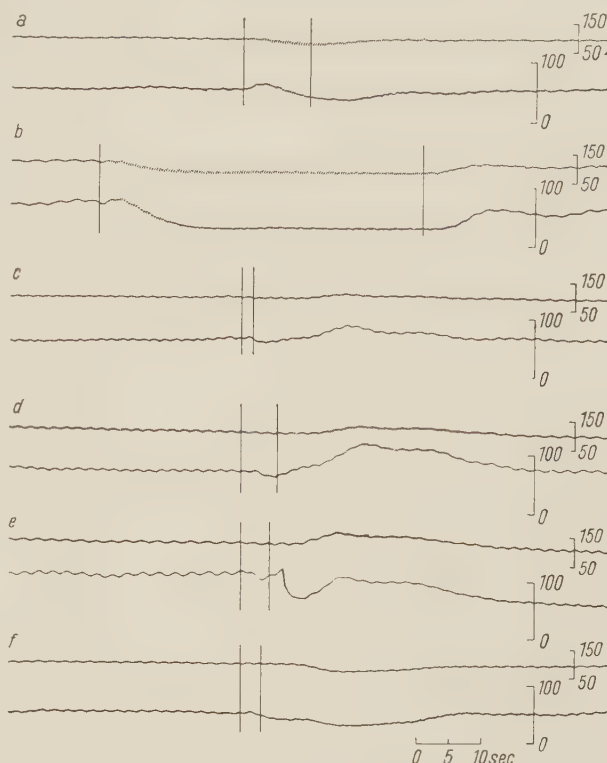


Fig. 1.—Cat 2.6 kg. Nembutal. Pairs of records of femoral (upper) and basilar (lower) pressures. Calibrations in mm Hg to the right. Signals from the femoral pressure transducer electronically damped. *a* and *b*: Between marks, electrical stimulation of the left cervical sympathetic trunk (2V in *a*, 4V in *b*) 33 pulses per sec. *c*, *d* and *e*: The effect of 1, 2 and 3 μ g respectively of 1-noradrenaline HCl injected into the left carotid artery through a cannula in the lingual artery. *f*: The effect of 1 μ g acetylcholine injected by the same route.

⁷ B. HOLMQVIST, D. H. INGVAR, and B. SIESJÖ, *Acta physiol. scand.* 40, 146 (1957).

A similar asymmetry was also found in the development of the carotid sinus. Thus, when clamping the common

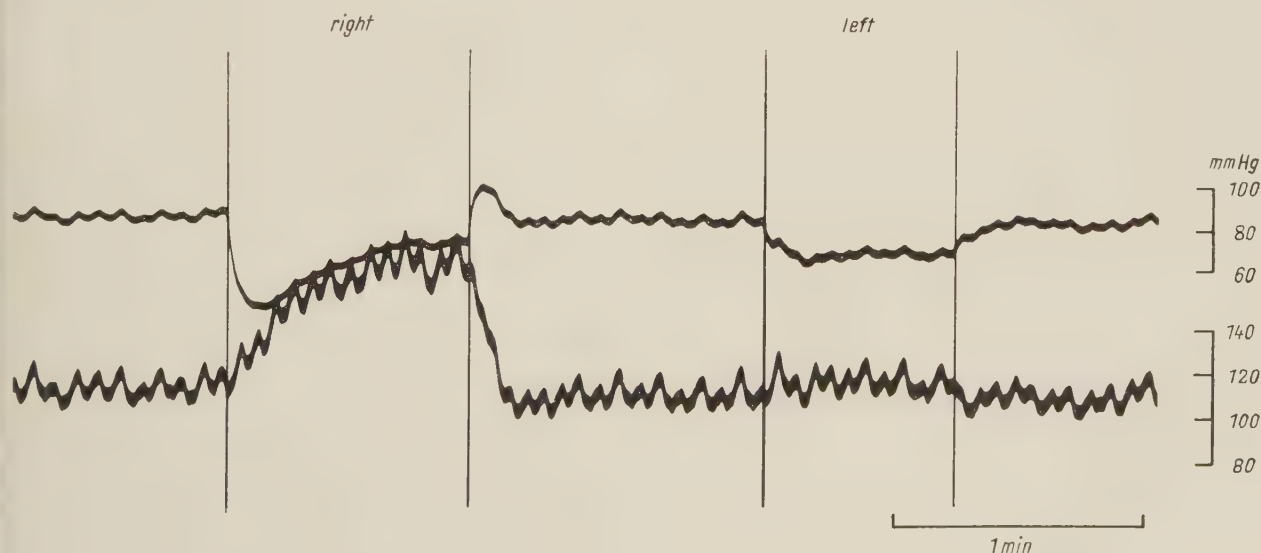


Fig. 2.—Cat 3.6 kg. Nembutal. Blood pressure of basilar (above) and femoral (below) arteries. Calibrations to the right. As indicated, right or left common carotid artery occluded. Note large pressure fall in the basilar artery coincides with strong carotid sinus reflex.

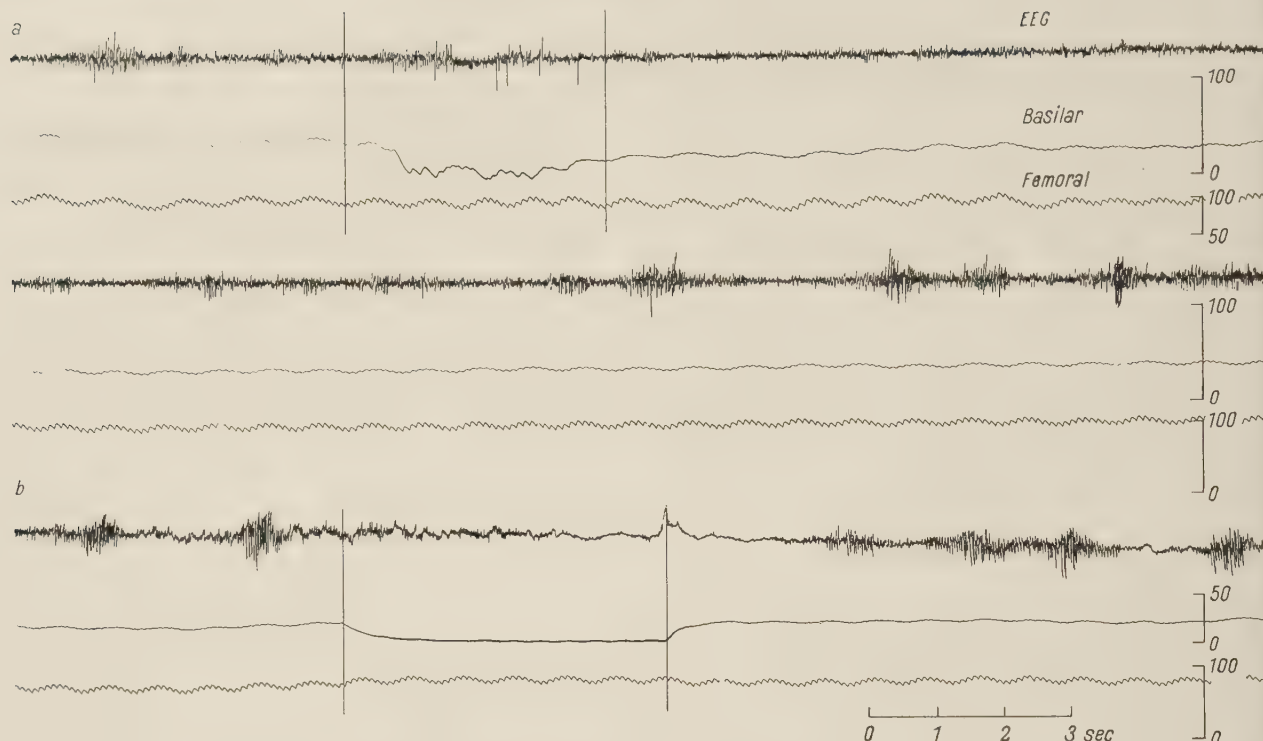


Fig. 3.—Cat 3.9 kg, Nembutal. Records from above: EEG from the cruciate region; basilar and femoral blood pressures (calibrations to the right). *a*: Two continuous records from the lightly anesthetized animal. Between vertical lines we tried to open the jaws and elicited a closing reflex and an 'arousal' reaction that is traced to the middle of the second record. Note irregular fall of basilar pressure. *b*: Cat now immobilized with 'Flaxedil'. Opening the jaws reduces basilar pressure to zero and EEG shows depressed cortical activity.

carotid artery was followed by decreased basilar pressure and reduced cortical blood flow, there was always a strong carotid sinus reflex raising the systemic blood pressure, whereas in those cases where the anastomoses were badly developed between the carotid artery on one side and the circle of Willis, the reflex from the sinus region on the same side was weak or absent (Fig. 2).

Arterial occlusion was also obtained after large doses of serotonin. In one experiment, this spasm was released by a serotonin antagonist⁸.

Wide separation of the animal's jaws caused a mechanical occlusion of the blood vessels between the carotids and the circle of Willis. In the intact anesthetized animal, jaw opening to the critical angle of 30° to 40° or more caused reflex closure, but in the curarized preparation where this reflex is abolished, complete arrest of arterial flow could be obtained by passive separation of the jaws. Electrical stimulation of the lingual nerve could evoke mouth opening to such an angle that the cerebral blood flow was significantly lowered.

The vertebral and basilar arteries are not large enough to replace the carotids during a strong carotid constriction. Therefore, all procedures that seriously reduced the carotid blood flow also influenced brain function. Slow waves, or even flattening of the EEG records could thereby be induced (Fig. 3). Increased tone in skeletal muscle, similar to that seen after anemic decerebration according to POLLOCK and DAVIS⁹, was also observed during constriction of the carotid arteries.

Conclusion.—The pressure drop in the extra- and intracranial portions of the arteries to the cat's brain is sometimes large enough significantly to influence the cerebral

blood flow. The fall in blood pressure in the arteries varies with arterial vasomotor activity as well as with changes in blood flow both inside and outside the skull. No evidence for special vasomotor activity in the carotid *rete* has been found. In view of previous reports in the literature, the finding of a considerable pressure drop in narrow arteries is not unexpected. Nevertheless, one would believe that the anatomical arrangement consisting of a number of arteries running in parallel to the circle of Willis would always permit adequate supply of blood to the brain. However, the present results show that a reduction in blood flow through one or two of these arteries cannot always be satisfactorily compensated for by increased flow in the remaining vessels. As a consequence, signs of depressed cerebral activity appear when the carotid arteries are markedly constricted.

The research reported in this document has been supported in part by the Office of Scientific Research of the Air Research and Development Command, United States Air Force, through its European office under Contract AF 61(052)-119. A grant from the foundation 'Therese och Johan Anderssons Minne' as well as support to N. W. from 'Signe och Ane Gyllenbergs Stiftelse' are gratefully acknowledged.

U. SÖDERBERG and N. WECKMAN

Nobel Institute for Neurophysiology, Karolinska Institute, Stockholm (Sweden), May 6, 1959.

Zusammenfassung

Bei der Katze kann der Druckabfall in den extra- und intrakraniellen Anteilen der Hirnarterien unter gewissen Bedingungen genügend gross werden, um die Hirndurchblutung in eindeutiger Weise zu beeinflussen. Schwankungen des Druckgradienten in den Arterien werden durch die arterielle Vasomotorik und Veränderungen der Durchströmung derjenigen Gewebe bedingt, die von den Aa. carotidae externae versorgt werden.

⁸ Methyl-lysergic acid diethylamide (MLD-41) and 5-hydroxytryptamine (serotonin) were kindly provided by Sandoz AG., Basel.

⁹ L. J. POLLOCK and L. DAVIS, J. comp. Neurol. 59, 377 (1930).

Investigations on the Influence of Aminoacetonitrile on Connective Tissue in the Hamster¹

It is well established that the connective tissues of weanling rats undergo significant degenerative changes following the administration of *Lathyrus odoratus* seeds. The causative agent in these seeds is β -aminopropionitrile. Other aminonitriles and especially aminoacetonitrile act in an even stronger way². The aminonitriles affect the mesenchymal tissues only, and the effect is most pronounced during certain periods of growth³.

In adult animals connective tissue growth was studied in healing wounds.

Functional and morphological studies were carried out on hamsters because it is possible to do microscopic studies *in vivo* in the cheek pouch of these animals during the experiment.

Methods.—39 young adult hamsters weighing 80–120 g were used in the experiments. They were all kept on the same diet. Longitudinal incisions were made on the back of the animals, 5 cm in length and always at the same distance from the spine. The wounds were closed with interrupted nylon sutures.

Crystalline aminoacetonitrile sulfate (AAN) was dissolved in a physiological saline solution and neutralized with sodium bicarbonate.

The investigation was divided into two different experiments. In the *first experiment* 12 hamsters were given 10 mg of the AAN in 1 ml solution by daily subcutaneous injections. 12 hamsters served as controls and received 1 ml of physiological saline solution only. Three animals from each group were killed with a large dose of nembutal on the 3rd, 5th, 7th, and 9th day after starting the experiment.

In the *second experiment* 8 hamsters were given 20 mg of AAN by daily subcutaneous injections, while 7 served as controls. They were all killed on the 7th day after the incisions.

All animals were given 150 μ C of ³⁵S-labelled sulfate (in physiological saline solution with 0.04% sodium sulfate) intraperitoneally 48 h before killing.

The mast cells of the cheek pouches were examined in each animal in nembutal anesthesia immediately before killing by a method previously described⁴.

¹ Aided by grants from the Danish State Research Foundation, the Danish Rheumatism Association, and the Sigrid Juselius Stiftelse, Finland.

² S. WAWZONEK, I. V. PONSETI, R. S. SHEPARD, and L. G. WIEDENMANN, *Science* **121**, 63 (1955).

³ I. V. PONSETI, *Clinical Orthopaedics*, number 9 (Lippincott, Philadelphia), p. 131.

⁴ O. WEGELIUS and G. HJELMMAN, *Acta path. microbiol. scand.* **36**, 304 (1955).

The tensile strength of the wounds was determined *in situ* and *in vivo* by the method described by SANDBLOM *et al.*⁵. The wound-areas were then carefully excised and examined for radioactivity by a method previously used⁶.

In the first experiment the femur bones of all animals were freed from muscles and tendons and the two groups compared.

In the second experiment the thoracic and abdominal aortae were carefully dissected free from the surrounding tissue; the arteries were pooled and dried and the concentrations of hexosamine and hydroxyproline were determined. This part of the experiment was repeated in a new series giving a total of 15 animals in each of the two groups. The hexosamine content was determined by the ELSON and MORGAN method⁷ as modified by DYRBYE⁸, and the hydroxyproline content by MARTIN and AXELRODS modification⁹ of NEUMAN and LOGANS procedure¹⁰.

Results.—*Tensile strength of the wounds:* The values of the treated group in the first experiment were lower than the controls at the 0.03 level of significance. The decrease was even more pronounced in the second experiment ($p < 0.001$) in which a larger number of animals received the double dose of AAN (Table I).

³⁵S-sulfate uptake of the wound tissue: The determination of radioactivity in the wounds showed no significant change in the uptake of ³⁵S-labelled sulfate during AAN administration. The values for the radioactivity in the second experiment are shown in Table I.

Mast cells observed in vivo in the cheek-pouches showed no morphological differences between the treated and the control groups. In both groups the mast cells were well-granulated and the number seemed to be similar in both groups.

The femur bones of the AAN-treated animals showed no change in the size, form or colour when compared with the controls.

The hexosamine and hydroxyproline concentration of the aortic tissue tended to show higher values in the AAN-treated group but the differences were not significant (Table II). The hexosamine:hydroxyproline ratio was the same in the two groups.

Discussion.—It is generally assumed that the changes in the tensile strength of healing wounds is caused first of all by changes in the amount and condition of collagen fibers¹¹. Our results of tensile strength determinations

⁵ P. SANDBLOM, P. PETERSEN, and A. MUREN, *Acta chir. scand.* **105**, 252 (1953).

⁶ E. MOLTKE, *Acta endocrinol.* **25**, 179 (1957).

⁷ L. A. ELSON and W. T. J. MORGAN, *Biochem. J.* **27**, 1824 (1933).

⁸ M. O. DYRBYE, *J. Gerontol.* **14**, 32 (1959).

⁹ C. J. MARTIN and A. E. AXELROD, *Proc. Soc. exp. Biol. Med.*, N.Y. **83**, 461 (1953).

¹⁰ R. E. NEUMAN and M. A. LOGAN, *J. biol. Chem.* **184**, 299 (1950).

¹¹ J. E. DUNPHY and K. N. UDUPA, *New England J. Med.* **253**, 847 (1955).

Table I

The tensile strength and the uptake of ³⁵S-labelled sulfate on the seventh day of wound healing in aminoacetonitrile poisoned hamsters

	Number of Animals	Tensile Strength	c.p.m./100 mg Wound tissue	Activity Ratio*
Controls	7	509 $\pm$ 29.4	78.8 $\pm$ 5.9	1.68 $\pm$ 0.15
Aminoacetonitrile (20 mg/daily)	8	163 $\pm$ 7.0	92.6 $\pm$ 14.4	1.39 $\pm$ 0.11
Significance		$P < 0.001$	none	none

* Activity Ratio: $\frac{\text{c.p.m./mg of wound tissue}}{\text{c.p.m./mg of skin.}}$

Table II

The hexosamine and hydroxyproline concentrations in the aortae of aminoacetonitrile-treated hamsters as compared with control animals (mg/g dry tissue)

	Number of Animals	Hexosamine	Hydroxyproline	Hexosamine hydroxyproline ratio
AAN-treated animals .	8	3.6	18.7	0.23
	7	5.9	21.7	
Control animals . . .	7	3.3	12.3	0.23
	8	3.6	18.8	

agree with those of others¹² and may be regarded as the functional result of retarded maturation of fibroblasts influenced by lathyrogenic agents^{13,14}. They are also in accordance with the experiment which indicates that aminopropionitrile reduces the incorporation of ¹⁴C-glycine into collagen or ground substance¹⁵.

The decrease in collagen content of healing wounds caused by AAN is probably caused neither by a change in the metabolism of the sulfomucopolysaccharides (Table I) nor in the total hexosamines. This is supported by others who found no change in the tissue hexosamine during such treatment¹³ nor any change in the uptake of ³⁵S-sulfate in the tissues as determined by autoradiographic methods¹⁶. The lack of significant change in the hydroxyproline content of the aortic tissues is explained partly by the fact that the animals were adults and partly by the difficulties in dissecting the aortae free of adventitia and surrounding loose connective tissue.

Mast cell reactions to lathyrogenic agents have not previously been studied in living animals. The lack of morphological changes in the mast cells of the cheek-pouches in the AAN-treated group suggests that they are not the target cells.

O. WEGELIUS*, E. MOLTKE, and M. O. DYRBYE**

Connective Tissue Research Laboratory, University Institute of the Medical Anatomy, Copenhagen (Denmark), May 8, 1959.

Zusammenfassung

Aminoazetonitril entfaltet eine deutliche Wirkung am Bindegewebe des Goldhamsters. Diese Wirkung äussert sich in einer Verminderung der Zugfestigkeit von Wundgewebe. Die Mastzellen zeigten keine morphologischen Änderungen. Sowohl die Anreicherung von Radio-schwefel im Wundgewebe wie die Konzentrationen von Hydroxyprolin und Hexosamin im Aortengewebe änderten sich nicht in signifikanter Weise.

¹² F. M. ENZINGER and E. D. WARNER, Lab. Invest. 6, 251 (1957).

¹³ J. E. MIELKE, J. J. LALICH, and D. M. ANGEVINE, Proc. Soc. exp. Biol. Med., N.Y. 94, 673 (1957).

¹⁴ J. V. HURLEY, E. STOREY, and K. N. HAM, Brit. J. exp. Path. 39, 119 (1958).

¹⁵ N. C. BRUEMMER, L. L. LALICH, and G. C. MUELLER, Proc. Soc. exp. Biol. Med., N.Y. 96, 340 (1957).

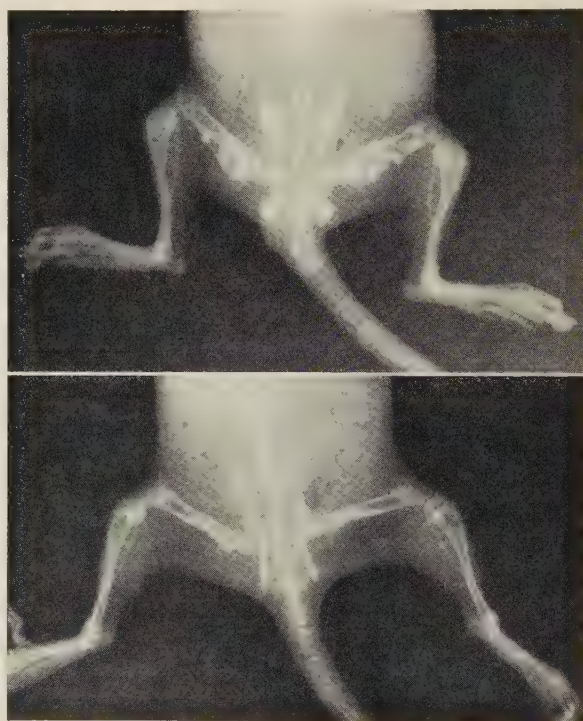
¹⁶ I. V. PONSETI, S. WAWZONEK, R. S. SHEPARD, T. C. EVANS, and G. STEARNS, Proc. Soc. exp. Biol. Med., N.Y. 92, 366 (1956).

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Iproniazid and Experimental Lathyrism¹

There is evidence that the metabolically active radical of the β -aminopropionitrile is the amino group. It must not be substituted², and in the physiological detoxication a nontoxic cyanoacetic acid is formed³. On the contrary, the cyan group can be replaced with the SH-group⁴.



Top: iproniazid-aggravated experimental lathyrism; Below: lathyrism produced by identical diet without iproniazid.

A hypothesis that the amino-oxidase is involved in the detoxication of β -aminopropionitrile was tested with rats (10 in each group) by adding iproniazid (Marsilid 'Roche') to the diet⁵ (430 mg in 1000 g of food), which contained

¹ This work forms a part of a program supported by Sigrid Jusélius Foundation. Prof. C. WEGELIUS and Prof. O. JÄRVI have placed to our disposal the facilities for x-ray studies and Mr. I. LINDGREN, cand. med., has given much valuable advice.

² T. E. BACHHUBER, J. J. LALICH, D. M. ANGEVINE, E. D. SCHILLING, and F. M. STRONG, Proc. Soc. exp. Biol. Med., N.Y. 89, 294 (1957).

³ F. M. STRONG, J. J. LALICH, S. H. LIPTON, H. W. SIEWERT, and J. T. GARBUIT, IV. Internationaler Kongress für Biochemie, Communication 8-83, Wien 1958.

⁴ W. DASLER, Proc. Soc. exp. Biol. Med., N.Y. 88, 196 (1955).

⁵ Modified from the diet previously described in Exper. 13, 495 (1957) by L. KALLIOMÄKI, M. YLI-POHJA, and E. KULONEN.

about 75% of sweet pea (*Lathyrus odoratus* Spencer). The amount of both β -aminopropionitrile and Marsilid thus depended on the amount of food consumed but their ratio was constant. The results are presented in the table and the aggravating influence of Marsilid was fully corroborated with x-ray examination.

Reserpine did not influence the iminodipropionitrile-induced 'neurolathyrism'⁶, which is claimed to be different from osteolathyrism. We believe that monoamino-oxidase is concerned only with the detoxication, but not with the osteolathyrism itself, since the inhibition of monoamino-oxidase does not cause similar symptoms. Therefore, a decrease in the metabolism of the natural substrates of monoamino-oxidase cannot be the cause of osteolathyrism. This conclusion rests on the assumption that monoamino-oxidase inhibition is the full mode of iproniazid action.

Diet (for 22 days)	Food consumed per day	Gain in weight per day		X-ray findings of lathyrism
		mean and s.d.	% of resp. <i>Pi.</i> sum-diet	
<i>Pisum sativum</i> . . .	3.2	1.47 $\pm$ 0.30	—	—
<i>Lathyrus odoratus</i> . .	3.1	0.88 $\pm$ 0.19	62.6	
<i>Pisum sativum</i> , with Marsilid	2.4	0.92 $\pm$ 0.25	—	—
<i>Lathyrus odoratus</i> , with Marsilid	1.3	0.35 $\pm$ 0.13	38.0	very severe

The conclusion remains that the harmful action of the β -aminopropionitrile is due to a metabolic interference through its amino group.

The detailed account on the whole experiment is under preparation.

K. JUVA, LEENA MIKKONEN,
TAINA TUOMINEN, and E. KULONEN

Department of Medical Chemistry, University of Turku (Finland), January 20, 1959.

Zusammenfassung

Iproniazid verschlimmert den experimentellen Lathyrismus bei der Ratte.

⁶ H. SELYE, Rev. canad. Biol. 16, 1 (1957).

Elektromyographische Untersuchungen
der Wirkung von LSD 25 auf die neuromuskuläre
Reizübertragung beim Kaninchen

Von zahlreichen Forschern ist das Vorhandensein eines Synergismus oder Antagonismus (je nach der Dosis) zwischen Serotonin (5-HT) und LSD 25 (Lysergsäurediäthylamid) diskutiert worden.

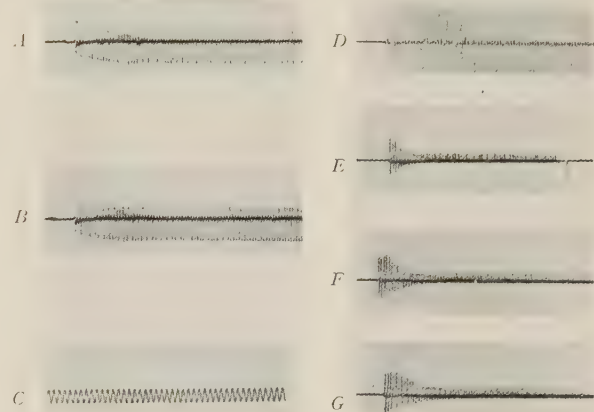
Frühere Versuche (SALA und PERRIS¹) bewiesen elektromyographisch eine geringe curareantagonistische Wirkung des 5-HT auf die neuromuskuläre Reizübertragung. Dieser Befund veranlasste uns zu untersuchen, ob auch mit LSD 25, allein oder in Verbindung mit 5-HT, eine ähnliche Wirkung erzielt werden kann.

¹ E. SALA und C. PERRIS, Il Neurone (Mantova) 6, 1 (1958).

Methode. Für die Versuche wurden 10 männliche Kaninchen mittleren Gewichts (1,750–2 kg) verwendet. Bei allen Untersuchungen wurde folgende, bereits geprüfte Technik angewandt: Stimulierung des *N. ischiadicus* mit kurzen Reizströmen von 100 Hz und Ableitung des muskulären Aktionspotentials der Flexoren der Pfote mittels Adrianscher Nadel. Registriert wurde mit einem Kathodenstrahl-Elektromyographen, wobei der Ausgang des Vorverstärkers (Offner 141) mit der horizontalen Ablenkplatte des Oszilloskops verbunden wurde. Die Oszilloskop-Bilder wurden mit einer 35-mm-Filmkamera mit konstantem Filmvorschub von 8 cm/s aufgenommen. Als Kriterium für die Bewertung des Wedenskyeffektes wurde bei allen Bestimmungen der prozentuale Wert der Amplitude der zehnten Schwingung im Verhältnis zur Amplitude der ersten Schwingung der jeweiligen Reizfolge genommen.

In verschiedenen Versuchsgruppen wurde eine eventuelle Wirkung des LSD 25 in diversen Dosierungen (0,05–0,1–0,15 mg i. v.) untersucht

- a) am Normaltier,
- b) am unter teilweise Curareblock stehenden Tier (D-Tubocurarin 0,09 mg/kg i. v.),
- c) vor und nach intravenöser Verabreichung von 5-HT (eine Ampulle² entsprechend 5 mg 5-HT und Kreatininsulfat als Doppelsalz).



- A–B Maximale Stimulation des Nervus ischiadicus mit Reizfolgen von 100/s. Ableitung von den Flexoren mittels Adrianscher Nadel. A = 1 min vor und B = 1 min nach der i.-v.-Injektion von 1 Ampulle LSD 25. Es ist zu beachten, dass das nach der Injektion aufgenommene Oszillogramm keine Änderung gegenüber der Normalkurve aufweist.
- D–E–F–G Anderer Versuch unter gleichen technischen Bedingungen.
- D Normaloszillogramm.
- E 2 min nach i.-v.-Injektion von 0,06 mg/kg D-Tubocurarin (beim 5. Reiz Abfall der Amplitude um 50%).
- F Verminderung des partiellen Curareblocks durch 5-HT (der Abfall von 50% verschiebt sich bis zum 10. Reiz).
- G Injektion von 0,1 mg LSD 2 min nach Injektion von 5-HT und 5 min nach jener von D-Tubocurarin (keine wahrnehmbaren Änderungen des 5-HT-Effektes).
- C Eichung für alle Oszillogramme: 50 Hz für die Zeit, 3000 mV für die Amplitude.

Resultate und Kommentar. Die Resultate können wie folgt zusammengefasst werden:

² Produkt der Firma VISTER, welches unter dem Namen «Antemovis» im Handel ist.

1. LSD 25 zeigte in den angewendeten Dosen beim Kaninchen keine curareähnliche Wirkung. Bis 20 min nach Verabreichung von LSD 25 konnten in den 3 angegebenen Dosierungen keine wahrnehmbaren Änderungen der Amplitude des muskulären Aktions-Potentials festgestellt werden.

2. LSD 25 hatte bei den unter partiellem Curareblock stehenden Tieren und in der angegebenen Dosierung keinerlei antagonistische Wirkung gegenüber Curare. Die Kurve, welche das Absinken und die allmähliche Wiederherstellung des muskulären Aktions-Potentials nach Verabreichung von 0,09/kg D-Tubocurarin wiedergibt, liegt höher als die entsprechende mittlere Kurve der Kontrolltiere.

3. LSD 25 hat endlich die geringe curareantagonistische Wirkung des 5-HT auf die neuromuskuläre Reizübertragung nicht beeinflusst, sei es unmittelbar vor oder nach i.v.-Applikation von Serotonin verabreicht worden.

4. Die negativen Resultate lassen sich vielleicht durch die Beobachtungen von THOMPSON, TICKNER und WEBSTER^{3,4} erklären, welche feststellten, dass LSD25 hauptsächlich bei Laboratoriumstieren einen geringen Einfluss auf die eigentliche Cholinesterase hat, während es elektiv die Pseudocholinesterase hemmt.

Sie bestätigen ferner die Möglichkeit, dass der Antagonismus oder Synergismus zwischen LSD25 und Serotonin sich nicht überall entfaltet.

C. PERRIS

Laboratorio di E. E. G. dell'Ospedale Psichiatrico Provinciale di Cremona, 24. April 1959.

Riassunto

In un lotto di conigli si è indagata, con tests elettromiografici, l'eventuale azione della LSD 25 a livello della giunzione neuromuscolare.

Dalle esperienze effettuate è emerso che la sostanza (alla dose di mg 0,05-0,1-0,15 endovena, in animali di kg 2 circa) non possiede nè un'azione curarosimile, nè un'azione anticurarica.

Si è potuto rilevare inoltre, con opportune ricerche che la LSD25 non interferisce, in alcun modo, sulla modesta azione anticurarica esercitata dalla serotonina e documentata in altra sede.

³ R. HS. THOMPSON, A. TICKNER und G. R. WEBSTER, *Biochem. J.* 58, 19 (1954).

⁴ R. HS. THOMPSON, A. TICKNER und G. R. WEBSTER, *Brit. J. Pharmacol. Chemiother.* 10, 61 (1955).

On the Decrement Function of an Action Potential in a Volume Conductor

In a recently published paper by HÅKANSSON¹, the potential field of an isolated frog muscle fibre surrounded by a volume conductor (Ringer solution) is investigated. It proved difficult to obtain a simple function, describing the decrease in amplitude of the action potential along a normal to the fibre. HÅKANSSON found that near the fibre ($y_0 < 0.15$ mm) there is an approximative agreement with a function $\sim -\log y_0$. At a greater distance a

function $\sim y_0^{-1.3}$ is more adequate. I have shown² that these difficulties can be overcome by introducing time (t) and impulse velocity ($v = \text{constant}$) into LORENTE DE NO's³ formula for the spread of potential in a volume conductor and using its Fourier transform. Thus

$$\Phi(x_0, y_0, t) = h \int \frac{\partial^2 V_e}{\partial x^2} \cdot \frac{dx}{\sqrt{y_0^2 + (x_0 - x - vt)^2}} \quad h = -\frac{\omega}{4\pi} \quad (1)$$

where Φ = potential at x_0, y_0 ; y_0 = normal distance between electrode and fibre; V_e = external spike potential; ω = cross section of the fibre. The fibre is extended in the x -direction.

After Fourier transformation² we get

$$\begin{aligned} \tilde{\Phi}(x_0, y_0, p) &= h \cdot \tilde{\Theta}(p) \cdot \tilde{\Omega}(p); \\ \tilde{\Theta}(p) &= \int_{-\infty}^{\infty} \frac{\partial^2 V_e}{\partial x^2} e^{-ipx} dx; \quad t = \tau - \frac{x}{v} \\ \tilde{\Omega}(p) &= \int_{-\infty}^{\infty} \frac{e^{ip\tau}}{\sqrt{y_0^2 + (x_0 - v\tau)^2}} d\tau = \frac{e^{ipx_0}}{v} K_0(p y_0/v) (-2). \end{aligned} \quad (2)$$

K_0 is a modified Bessel function, the graph of which is drawn in Figure 2. In eq. 2 $\tilde{\Theta}(p)$ depends only on the shape of the potential and $\tilde{\Omega}(p)$ only on the geometry at the recording and the velocity of the impulse. From eq. 2 it is evident that every frequency component follows its own decrement curve, which is a K_0 in its own scale, since the frequency is a factor in the independent variable of K_0 .

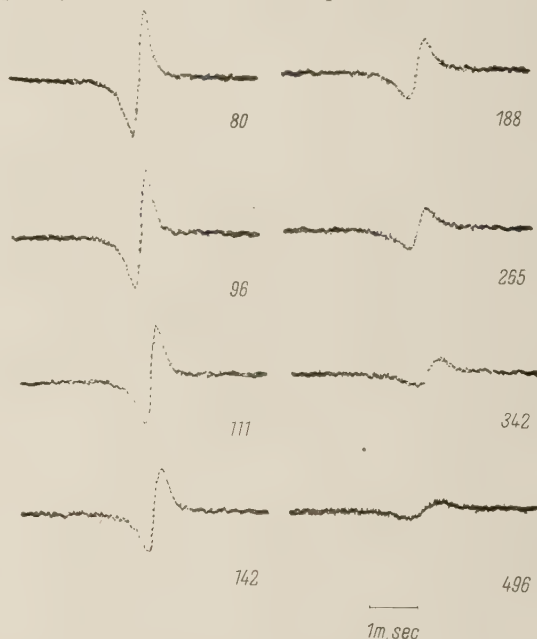


Fig. 1.—Action potentials from an isolated frog muscle fibre of radius 34μ in a Ringer solution. The number on each curve denotes the distance between the fibre and the electrode (y_0) in μ .

Therefore, a higher frequency has a steeper decrement than a lower one, which explains the change of shape of the impulse with increasing distance. Theoretically the velocity of the impulse is uniquely determined by the

² C. E. T. KRAKAU, *Kgl. Fys. Förh.* 27, 177 (1957).

³ R. LORENTE DE NÓ, *Studies Rockefeller Inst. Med. Res.*, New York 132, 384 (1947).

¹ C. H. HÅKANSSON, *Acta physiol. scand.* 39, 291 (1957).

amplitude of a single frequency at two different points at known distances from the fibre. As an illustration, an experiment performed by HÅKANSSON has been worked out. The action potential has been recorded at 8 different distances (80–496 μ) from the centre of a frog muscle fibre in Ringer solution (Fig. 1). The fibre is considered infinitely thin in relation to the electrode distance. For the 2 nearest points (80 and 96 μ) this means an error, which is estimated at as much as 10% for the highest frequencies. With increasing y_0 the error falls rapidly. During the experiment the value for the velocity was determined at 2.0 m/sec.

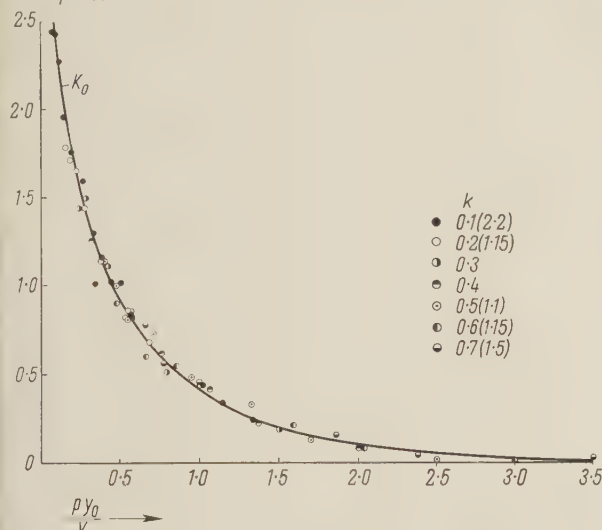


Fig. 2.—Full drawn line: the graph of the function K_0 (py_0/v). The number k is related to frequency p by $k = p/2\pi \cdot 4250$. A series of similar dots represents the decrease of a certain frequency (p) with increasing distance (y_0). In brackets: the factor necessary for curve fitting of the amplitude values.

The amplitudes of the curves (Fig. 1) have been measured at c:a 50 equidistant points on each. The absolute value of integral (2) has been calculated for seven frequencies in the range 425–2975 cps by means of a digital computer. Simpson's rule has been applied. The decrease of the amplitude for each frequency with increasing y_0 has to follow the K_0 . The values for each frequency have thus been multiplied by a common factor so as to fit the same K_0 . These factors are given in brackets in Figure 2. By means of this adjustment, the decrement curve for every frequency is brought into overlapping continuity with the following, and they all seem to follow a K_0 function well. However, the scale of K_0 that fits best is obtained by putting $v = 2.57$ m/sec instead of 2.0 found at direct measuring. This discrepancy may be explained by a.o. the size of the electrodes (30–50 μ tip diameter), which reduces the exactness of the position determinations. The example demonstrates a consequence of LORENTE DE NÓ's field equation, namely that the decrement of the action potential is determined not only by the geometry at recording but also by the impulse shape and velocity.

C. E. T. KRAKAU

Eye Department, University of Lund (Sweden), March 16, 1959.

Zusammenfassung

Eine Modifikation der Feldgleichung von LORENTE DE NÓ wird einer Fourier-Transformation unterworfen. Dabei zeigt sich, dass das Dekrement einer jeden Frequenz eine K_0 -Funktion in ihrem eigenen Maßstab darstellt. Die theoretische Dekrementkurve stimmt gut mit der experimentell eingearbeiteten überein.

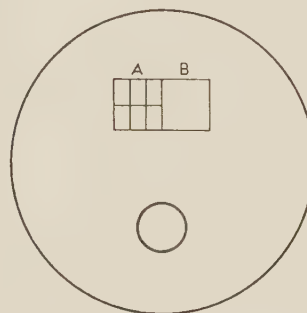
Determination of the Influence of Size in the Differentiation of an Isolate

Introduction. The significance of size in tissue differentiation is well known from various experiments.

DRAGOMIRROW¹ observed a failure of the pigmented epithelium of the eye to regulate into a cup if the isolate fell below a certain size. LOPASCHOV² found that an increase in the amount of head mesenchyme of an amphibian gastrula gave rise to a complexity of differentiation, whereas, one or two fragments developed into striated muscles. WEISS and AMPRINO³ also found that below a certain size, there would be no differentiation of the pre-scleral mesenchyme into cartilage in the chick.

Results of ANDRES⁴ and BERRILL⁵ and others also show that the degree of differentiation is dependent upon the mass of tissue.

GROBSTEIN^{6,7} and GROBSTEIN and ZWILLING⁸ made an extensive study of the nervous tissue differentiation in the mouse embryonic shield and in the chick blastoderm. They found a decrease in the percentage of neural differentiation with a decrease of size of the explants. GROBSTEIN⁶ observed that, when the fragmentation was carried out to 1/16 parts, differentiation was virtually eliminated with a 'dispersed cluster' and the percentage of neural differentiation was increased when these 1/16 parts were made into a 'close cluster'.



Mid gastrula of *Triturus alpestris* (A) represents the area which was subsequently cut into smaller bits and these are represented by horizontal and vertical lines. (B) represents the same area on the other side of (A). It was not cut into smaller parts and treated as the control.

It was decided to conduct experiments similar to GROBSTEIN and ZWILLING's⁸ with amphibian presumptive neural plates by cutting a definite part of it into 1/2, 1/4, and 1/6 to determine the critical mass of these isolates to undergo neural differentiation. Some of these parts were left intact as controls.

Technique and experiments. A square piece of the presumptive neural plate (0.5 × 0.5 mm) was excised from *Triturus alpestris* gastrulae with rounded blastopore (comparable to 13C stage of *T. pyrrhogaster*, OKADA and ICHIKAWA⁹) as shown in the Figure (A). It was carefully

¹ N. DRAGOMIRROW, Roux' Arch. 126, 636 (1932); 129, 522 (1933).

² G. LOPASCHOV, Biol. Zbl. 55, 606 (1935).

³ P. WEISS and P. AMPRINO, Growth 4, 245 (1940).

⁴ G. ANDRES, J. exp. Zool. 122, 507 (1953).

⁵ N. J. BERRILL, Growth and form (Oxford Univ. Press, England 1945).

⁶ C. GROBSTEIN, J. exp. Zool. 120, 437 (1952).

⁷ C. GROBSTEIN, Ann. N. Y. Acad. Sci. 60, 1095 (1955).

⁸ C. GROBSTEIN and E. ZWILLING, J. exp. Zool. 122, 259 (1953).

⁹ Yo. K. OKADA and M. ICHIKAWA, Jap. J. exp. Morph. No. 3 (1947).

freed from underlying mesoderm and then cut at random into 1/2, 1/4, and 1/6 parts. A similar square piece from the other side (Fig. B) was left intact as control. Sterile Holtfreter buffered (after DEUCHAR¹⁰) was used throughout the experiments and this contained a mixture of 15,000 i.u./l of Penicillin and Streptomycin. These small isolates, together with the controls, were cultured for 48 h. During this period, some of the isolates lost quite a number of cells and were rejected. The healthier ones were wrapped up either with *Triturus* or with *Xenopus* ectoderm and cultured for another 72 h. *Xenopus* ectoderm was mostly used in these experiments. The materials were fixed in Bouins. Sections were cut at 10 μ and stained either with celestine blue or with celestine blue and eosin.

Results and discussion. The amount of differentiation of the isolates varied from neural palisade to neural tube. This is shown in the following Table.

Size of the isolate	Total number of cases	Number of cases with differentiation	% of different pieces
1	7	7	100.0
1/2	21	18	86.0
1/4	31	22	71.0
1/6	25	17	68.0

It is clear from the small series of experiments that neural differentiation gradually dropped with the decrease in the size of the isolate down to the limiting value of 1/6. This size allowed the isolate to remain viable and undergo differentiation. Fragmentation beyond 1/6 was rather difficult as nearly all the fragments disintegrated soon after they were made.

GROBSTEIN⁷ observed that with the mouse and chick materials 1/8 was the 'critical mass' and 1/16 part never achieved this when left alone, but underwent neural differentiation when combined in 'close cluster'.

It seems from the present experiments that, in *T. alpestris* material, 1/6 is the 'critical mass' to undergo neural differentiation.

Acknowledgements. I am grateful to Professor C. H. WADDINGTON for his encouragement and for giving me all laboratory facilities in the Institute of Animal Genetics, Edinburgh, to Dr. F. BILLETT for valuable discussions and finally to the Calcutta University for a T. N. Palit foreign scholarship.

S. K. BRAHMA

Department of Zoology, Bangabasi College, Calcutta (India), March 31, 1959.

Résumé

On a détaché de l'ectoderme présomptif neural de mi-gastrulae de *Triturus alpestris* des lambeaux de grandeur définie qui ont été sectionnés au hasard en fragments d'une demi, d'un quart et d'un sixième, puis emballés dans de l'ectoderme de *Xenopus* ou *Triturus* et mises en culture dans la solution d'Holtfreter. Il a été constaté que le % des cas où une différenciation neurale s'est produite décroît avec la taille du fragment.

¹⁰ E. DEUCHAR, J. exp. Biol. 30, 18 (1953).

The Effect of Age on the Responses of Animal and Plant Tissues to Metabolic Inhibitors

An observation that the respiratory responses of tissues to metabolic inhibitors showed similar changes with age in plants and animals led to a series of experiments summarised below.

(i) Respiration rates of slices from the brains of 1-3-day and 14-17-month old rats were determined in the presence and absence of different metabolic inhibitors (Table I and II). The rats were killed by a blow on the neck, the brain dissected out, halved, and weighed. Each half was cut into thin slices and placed in one of a pair of Warburg vessels containing Krebs-Ringer solution¹ plus 2% glucose, and one of which contained in addition the dissolved inhibitor. Oxygen uptakes were determined at 37.2°C by conventional methods¹. Studies using cyanide were carried out according to ROBBIE². Two sets of experiments were performed, one in 1956 using 14-month old rats (Table I) and one in 1958 using 17-month old rats (Table II).

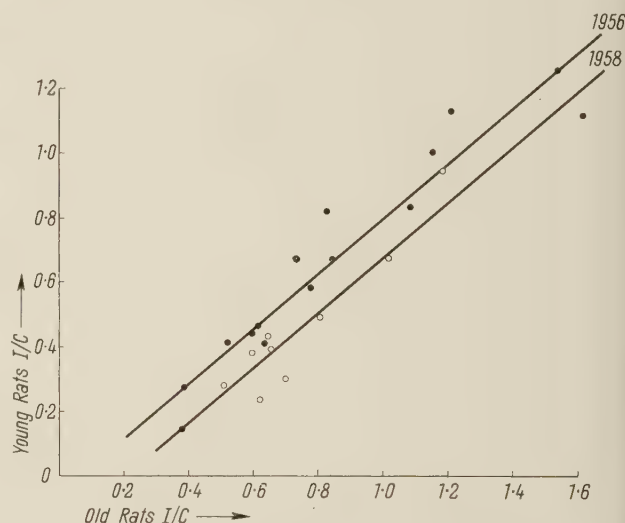


Figure 1.—The ratio (I/C) of respiration rate of young and old rat brain in presence (I) or in absence (C) of various respiratory inhibitors.

A statistical analysis of the differences in oxygen uptake for control (C) and inhibited (I) units was considered. The variability of this difference over the set of inhibitors and concentrations used, was greater for young rats than for old rats. Consequently separate within-animal standard errors, based on 23 degrees of freedom, are presented for testing the overall differences ($C - I$). The mean values for all 24 trials are presented in Table III. The presence of inhibitors reduces markedly the oxygen uptake of young rat brain tissue. This contrasts with the much smaller and non-significant effect of inhibitors on old brain, where the mean oxygen uptake is much lower than that of young rat brains.

When the ratio of 'inhibitors' to 'control' respiration rates (I/C) was calculated for each concentration of each inhibitor for old rats (denoted by x) and for young rats (denoted by y) and regression lines of the form $y = a + bx$ were fitted for the 1956 and 1958 sets of data (Fig. 1), it was found that a pooled analysis could

¹ W. W. UMBREIT, R. H. BURRIS, and J. F. STAUFFER, *Manometric Techniques* (Burgess Publish. Co., Minneapolis 1957).

² W. A. ROBBIE, *Methods in Medical Research* (Edit. V. R. POTTER, Year Book Pub., Chicago 1948).

Table I
The effect of various inhibitors on the respiration of old and young rat brains compared. Experiments of 1956

Inhibitor	Molar Concentration		Oxygen μl/g fresh weight/h		I/C	
			Old	Young	Old	Young
Cyanide	5 × 10 ⁻⁴	C	104	324	0.78	0.58
		I	81	189		
	5 × 10 ⁻⁴	C	291	458	0.38	0.14
		I	111	64		
	10 ⁻⁴	C	231	824	1.62	1.11
		I	375	913		
Azide	10 ⁻⁴	C	399	959	1.54	1.25
		I	614	1198		
	10 ⁻³	C	233	440	0.83	0.82
		I	194	360		
	10 ⁻³	C	238	470	0.85	0.67
		I	202	316		
Malonate.	5 × 10 ⁻⁴	C	171	223	0.64	0.41
		I	110	92		
	2 × 10 ⁻²	C	189	423	0.39	0.27
		I	73	115		
o-Phenanthroline 2:4	10 ⁻³	C	436	482	0.52	0.41
		I	225	197		
DNP	10 ⁻³	C	223	438	0.62	0.46
		I	138	201		
	10 ⁻⁴	C	310	518	1.09	0.83
		I	338	429		
	3 · 3 × 10 ⁻⁵	C	247	151	1.21	1.12
		I	298	169		
Diethyldithiocarbamate	2 × 10 ⁻⁵	C	282	461	1.16	1.00
		I	327	461		
	2 × 10 ⁻³	C	281	613	0.74	0.67
		I	209	413		
Iodoacetate	2 × 10 ⁻³	C	294	526	0.60	0.44
		I	175	231		

Table II
The effect of various inhibitors on the respiration of old and young rat brain compared. Experiments of 1958

Inhibitor	Molar Concentration		Oxygen μl/g fresh weight/h		I/C	
			Old	Young	Old	Young
Cyanide	2 × 10 ⁻⁴	C	230	634	0.65	0.43
		I	149	274		
	10 ⁻⁴	C	191	633	0.81	0.49
Azide	2 × 10 ⁻⁴	I	154	313		
		C	191	386	0.51	0.28
		I	98	109		
Malonate.	10 ⁻²	C	215	653	0.70	0.30
		I	149	193		
o-Phenanthroline . . .	10 ⁻³	C	152	682	0.66	0.39
		I	101	268		
2:4 DNP	2 × 10 ⁻⁴	C	216	572	0.60	0.38
		I	131	217		
		C	207	687	1.19	0.94
Diethyldithiocarbamate	2 × 10 ⁻³	I	246	642		
		C	266	471	0.62	0.24
		I	165	111		
Iodoacetate	2 × 10 ⁻³	C	317	743	1.02	0.67
		I	322	499		
Na Fluoride	10 ⁻³	C				
		I				

be carried out on the two sets of data and further that the slopes of the two lines did not differ significantly. The distance between these parallel lines was then tested and found to differ significantly from zero. Thus the situation reduced to having two parallel lines of the same slope, the equations for which are:

$y = 0.853x - 0.059$ for 1956;
 $y = 0.853x - 0.183$ for 1958.

and

The standard error of the regression coefficient, 0.853, is ± 0.0595.

It was found that when such brains were homogenised,

Table III
Mean oxygen uptake of old and young rats in presence (I) and absence (C) of various inhibitors (μl/g fresh weight/h)

	C	I	C-I
Old rats	246	208	38 ± 19.2
Young rats	532	332	200 ± 35.7

the differences in respiration rate and response to inhibitors largely disappeared, a result in accord with those of WEINBACH and GARBUS³ and of REINER⁴ but in contrast to these workers, a 'physiological' salt solution was used. The homogenizing would however still produce an unnatural environment in which the enzymes were active.

(ii) Since the recorded oxygen uptake in the experiments were somewhat lower than those given by KREBS⁵, testis tissue was chosen because the seminal tubules would easily separate from one another, and the thickest tubules present would not exceed the 0.3 mm dimensions critical for oxygen diffusion¹.

Four concentrations of cyanide, azide, and 2:4 dinitrophenol were used. The oxygen uptakes and I/C ratios are presented in Table IV. The data allowed a test of the difference between the within- and between-inhibitors regression. These two regressions do not differ significantly and thus the degree of association between the I/C ratios for young and old material is unaffected by the inhibitor factor. It was found that the situation could be adequately

³ E. C. WEINBACH and J. GARBUS, *Nature* 178, 1225 (1956).
⁴ J. M. REINER, *J. Gerontol.* 2, 315 (1955).
⁵ H. A. KREBS, *Biochim. Biophys. Acta* 4, 249 (1950).

represented by the total regression equation:
 $y = 0.051 + 0.793x$ (S.E. ± 0.1023).

It could be concluded therefore that the difference between old and young brains could not be attributed entirely to thickness of slice nor differences in permeability to various inhibitors.

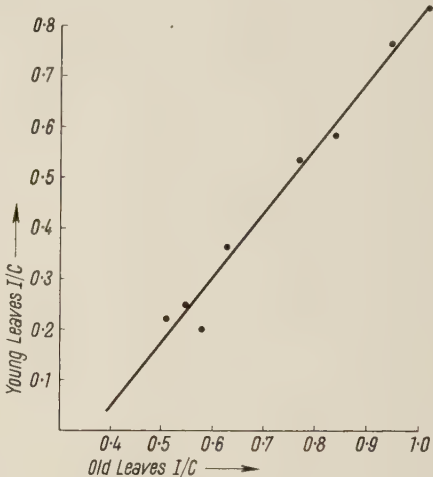


Figure 2.—The ratio (I/C) of respiration rate of young and old tomato leaves in presence (I) and in absence (C) of various respiratory inhibitors.

(iii) Similar studies were carried out using the uppermost fully expanded leaf and the 6th leaf of a series of uniform tomato plants, grown in water culture in a greenhouse⁶. The I/C ratios obtained from these leaves are

⁶ I. R. MACDONALD and P. C. DEKOCK, *Physiol. Plant.* 11, 464 (1958).

Table IV
The oxygen uptake of old and young rat testis in presence (I) or absence (C) of various concentrations of cyanide, azide, or 2:4 dinitrophenol (DNP)

Inhibitor	Molar Concentration		Oxygen uptake μl/g fresh weight/h		I/C	
			Old	Young	Old	Young
Cyanide	2×10^{-4}	C	415	430	0.52	0.55
		I	215	236		
	2×10^{-4}	C	400	415	0.28	0.22
		I	112	93		
	1×10^{-4}	C	400	415	0.35	0.33
		I	140	136		
	1×10^{-5}	C	309	415	1.04	0.86
		I	319	355		
	2×10^{-4}	C	364	510	0.45	0.52
		I	165	265		
Azide	2×10^{-4}	C	375	490	0.53	0.47
		I	200	230		
	1×10^{-3}	C	375	490	0.43	0.36
		I	160	175		
	1×10^{-4}	C	375	490	0.60	0.53
		I	225	260		
	2×10^{-4}	C	276	516	0.57	0.41
		I	158	213		
	2×10^{-4}	C	330	357	0.36	0.25
		I	120	90		
DNP	1×10^{-4}	C	330	357	0.38	0.41
		I	125	145		
	5×10^{-5}	C	330	357	0.48	0.45
		I	160	162		

Table V
The effect of varying concentrations of different inhibitors on young and old tomato leaf respiration

Inhibitor	Molar Concentration		Oxygen uptake μl/100 mg dry weight/h		I/C	
			Old	Young	Old	Young
Cyanide	5 × 10 ⁻⁴	C	2.12	3.13	0.58	0.20
		I	1.23	0.63		
	25 × 10 ⁻⁴	C	1.96	2.90	0.51	0.22
		I	1.00	0.63		
DNP	1 × 10 ⁻⁴	C	1.52	3.01	0.84	0.58
		I	1.27	1.74		
	1 × 10 ⁻³	C	1.83	2.87	0.55	0.25
		I	1.01	0.72		
CO:O ₂ (80:20)	—	C	1.64	2.90	1.02	0.83
		I	1.68	2.40		
	—	C	1.80	2.91	0.77	0.53
		I	1.38	1.53		
o-Phenanthroline . . .	1 × 10 ⁻³	C	1.58	3.00	0.95	0.76
		I	1.50	2.29		
Sodium Azide	1 × 10 ⁻³	C	1.59	2.78	0.63	0.36
		I	1.00	1.01		

recorded in Table V. The regression equation for these points is: $y = 1.267x - 0.460$ as shown in Figure 2. The standard error of this regression coefficient is ± 0.0742 . The significance of the proportional responses of old and young leaves has already been discussed⁶⁻⁸. In these papers it is suggested⁷ that the ratio of total Phosphorus to Iron content and Potassium to Calcium in the tissues gives some indication of the metabolic state of the plant cell. In⁶ the relationship between Phosphorus content and available Iron and the effect of this Iron on the metabolism of the cell is discussed and also the effect of respiration inhibitors.

It is evident that similar trends are obtained for both animal and plant tissues and that the following generalisations would be valid for both groups:

The proportionality of response of old and young tissues to inhibitors, irrespective of their nature, site of action or concentration, indicates

- (a) that the metabolism of the cell is a regulated entity and not a series of separate processes each of which has its own limiting factor,
- (b) that the balance between the various processes changes with ageing of the organism and to some extent can be used to reflect the age of the organism,
- (c) that no metabolic system is lost or new system introduced during the life of the organism.

Supporting evidence for the above views can be found in published analyses of animal tissues (LOWRY and HASTINGS⁹, LANSING¹⁰, GANS¹¹, PEARSON¹²).

C. MUIR, L. L. DEKOCK,
P. C. DEKOCK, and R. H. E. INKSON

Zoology Department, University of Aberdeen and Macaulay Institute for Soil Research, Aberdeen (Scotland), March 16, 1959.

⁷ P. C. DEKOCK and A. HALL, *Plant Physiol.* 30, 293 (1955).
⁸ P. C. DEKOCK and R. I. MORRISON, *Biochem. J.* 70, 272 (1958).
⁹ O. H. LOWRY and A. B. HASTINGS, *Cowdrey's Problems of Ageing* (The Williams & Wilkins Comp., Baltimore 1952).
¹⁰ A. I. LANSING, *Biol. Bull.* 82, 392 (1942).
¹¹ H. GANS, *Brain* 46, 178 (1923).
¹² P. B. PEARSON, *J. biol. Chem.* 106, 1 (1934).

Zusammenfassung

Junge Gewebe von Ratten zeigen eine grössere Empfindlichkeit gegenüber verschiedenen Konzentrationen von Atmungsinhibitoren als alte Gewebe. Ähnliche Resultate wurden bei der Einwirkung derselben Inhibitoren auf junge und alte Blätter von Tomatenpflanzen erzielt. Die Unterschiede sind statistisch signifikant. Es wird angenommen, dass keine neuen metabolischen Systeme während des Lebens eines Organismus gebildet werden, sondern dass während des Alterns nur eine Verschiebung im Gleichgewicht dieser Systeme eintritt.

PRO EXPERIMENTIS

A Simplified Method of Preparing
Buffered Egg-Yolk Extracts
Used as Diluter for Human and Bull Semen

Developing a method for the study of the kinetics of the killing of spermatozoa, we came across the need for an optically empty diluter which does not in any way impair the motility or the chance of survival of the spermatozoa. Considering the very advantageous properties of egg-yolk diluters, we set out with the idea of preparing a buffered extract of egg-yolk with the above-mentioned properties. Without knowledge of the work being done in this field by RIKMENSPOEL¹, we applied similar methods, i.e. ultra-centrifugation and repeated ultrafiltration. Although the result was satisfying, this mode of preparing the diluter appeared too laborious and expensive to permit its extensive use. However, during this work it was observed that a suspension of egg-yolk in citrate buffer settles down spontaneously, giving a slightly opaque supernatant.

Mode of preparation. One volume of egg-yolk, thoroughly freed from egg-white and yolk membrane, is stirred for 5 min with 2 volumes of citrate buffer (*M* 0.986, pH 7.4)

¹ R. RIKMENSPOEL, *Exper.* 13, 124 (1957).

Table I

Amount of nitrogen and phosphorus in different samples of diluter

Sample No.	mg N/ml	mg P/ml
1	7.37	1.67
2	7.36	1.66
3	7.36	1.70
4	7.34	1.69

being 0.015 *M* as to sulphanilamide, and containing 335 IU of benzylpenicilline (sodium salt, Glaxo) per ml. After thorough mixing, the suspension is left at + 2.0°C for 48 h, after which time the supernatant is pipetted off.

Properties. The liquid obtained shows a yellowish colour and a feeble turbidity when inspected in a thin layer. Yolk particles of a size comparable with that of the sperm heads are very scarce. Smaller particles with a diameter below 1 μ are abundant, but do not consistently disturb the dark background at darkfield illumination. The pH varies between 6.90 and 7.00. Analyses of the content of N and P show small variations from sample to sample (Table I). This diluter may be stored at + 2°C for weeks without losing its properties.

Table II

Percentage of mobile bull spermatozoa kept at + 4°C in diluter

Samples No.	1		2		3	
H in diluter	4	24	4	24	4	24
Spermatozoa giving 'scat- ing tracks' ²	56	37	65	55		
Spermatozoa progressing without rotation	23	32	23	25		
Spermatozoa swimming in circles	1	2	—	—		
Movement unclassified . .	1	2	1	1		
Total mobile	81	73	89	81	81	79

So far we are only able to judge the effect of this diluter on the survival of spermatozoa from observations on mobility.

Such observations on second ejaculates from three different bulls are presented in Table II. The semen was diluted 1:100 immediately after being collected, and the mobility registered after 4 and 24 h using a special photographic method which will be described elsewhere. In a similar experiment with human spermatozoa, the semen was diluted ten times only after 4 h, the semen being kept at room temperature before dilution. During the following 20 h at + 4°C the percentage of the mobile spermatozoa decreased from 81 to 70.

P. E. LINDAHL and K. WEDIN

Institute of Zoophysiology, University, Uppsala, May 4, 1959.

Zusammenfassung

Es wird eine vereinfachte Methode zur Herstellung eines isotonischen zitrathaltigen Extraktes von Eidotter

beschrieben, der frei von Dotterteilchen grösser als 1 μ ist. Die Konstanz der Zusammensetzung bei wiederholter Herstellung dieses Extraktes sowie sein Einfluss auf die Motilität von Spermatozoen werden untersucht.

PRO EXPERIMENTIS

A Simple Objective Method for Determination of the Glare Effect

(Preliminary report)

On the whole, there are two ways in which glare could affect the visual ability. One is caused by a lowered foveal sensitivity when a strong light falls into the eye (adaptive or retinal type). The other type appears when the eye is exposed to a light falling from the side. In this case, there is a diffuse diffraction and scattering of light in the optic media throwing an extra veil of light on the image of the object whereby the brightness of the object as well as of its surroundings is heightened (diffuse or veiling type). (For a survey of the physiology of glare, see e.g. GOLDMANN¹.)

The effect of glare on vision has generally been determined by subjective methods, based on the report of a test person. A more objective method of determining the visual ability is to make use of optokinetic nystagmus, registered electro-nystagmographically. In view of the fact that investigations of this type have not been carried out previously for the purpose of studying the glare effect, this preliminary report has been made with the object of drawing attention to a new and simple method which, in addition, happens to be as objective as could reasonably be expected.

Method. Optokinetic nystagmus was produced by projecting on to a screen, in the shape of a semi-circular cylinder, a number of vertical black and white stripes of equal width which were caused to move horizontally across the screen. The illumination was 20 lux. A detailed description of the apparatus will be found in a paper submitted by BLOMBERG². The eye movements in the horizontal plane were registered electro-nystagmographically in the customary manner. Use was also made of vertical electrodes to note any possibly occurring blinks in the course of the experiment. The movement of the stripes was adjusted at optimum speed, i.e. the speed at which maximum frequency and highest amplitude of optokinetic nystagmus were achieved. The experiments took place in a darkened room.

In order to produce glare of the adaptive type, a photographic flash (duration 1/1000 s) was triggered behind the individual undergoing the experiment. The flash was directed towards the ceiling. For the purpose of preventing the flash from causing consternation, with eye-shutting or blinking as a natural consequence, the test subject was told beforehand of the sequence of procedure. Attempts were also made to bring about diffuse glare by transmitting a beam of light from an ordinary torch held at a distance of about 20 cm from the eye in the orbital plane, from directly ahead, and from the side, at an angle of about 45 degrees to the visual axis. The other eye was held shut during this experiment.

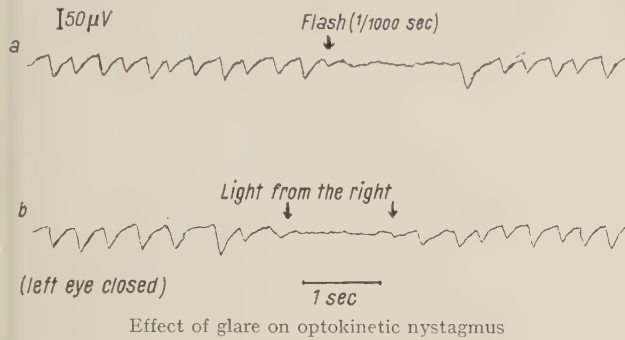
Results.—It was found that the glare caused by the flashlight exposure had the effect of abolishing optokinetic

² L. ROTSCHILD, *Mammalian Germ Cells*, CIBA Found. Symp. (London 1953), p. 122.

¹ E. GOLDMANN, *Bull. Schweiz. elektrotechn. Ver.* 41, 751 (1950).

² L.-H. BLOMBERG, *The 'optokinetic fusion limit'. A study in 56 healthy persons.* (To be published.)

nystagmus for approximately $1\frac{1}{2}$ s. The same effect was produced on experiments with diffuse glare. Optokinetic nystagmus then ceased for as long as the eye was affected by the exposure (see Fig. *a* and *b*).



Effect of glare on optokinetic nystagmus

Comments.—The fact that optokinetic nystagmus abolished tends to show that the retinal sensitivity was reduced

to such an extent that the moving stripes on the optokinetic screen could no longer be seen. The eye movement evoked by the optic stimulus therefore ceased. The method introduced in the above may be characterised as highly objective and rather appropriate for more exhaustive studies of the glare effect (for example under the influence of various pharmaceuticals).

L.-H. BLOMBERG

Clinical Neurophysiological Laboratory, Sahlgrenska Sjukhuset, Gothenburg (Sweden), April 17, 1959.

Zusammenfassung

Mit Hilfe des optokinetischen Nystagmus und Elektro-nystagmographie kann man den Blendungseffekt auf das Sehvermögen bestimmen. Während der Blendung hören die optokinetischen Augenbewegungen auf. Der Effekt eines photographischen Blitzes (1/1000 s) und von schräg in das Auge fallendem Licht wurden studiert.

Informations - Informationen - Informazioni - Notes

STUDIORUM PROGRESSUS

Effect of Single and Chronic Thyroxine Injection on Fatty Acid and Cholesterol Synthesis in Mice¹

By PAOLA MARCHI² and J. MAYER³

The action of thyroid hormone treatment on fatty acid synthesis from acetate has been previously investigated by SPIRITES, MEDES, and WEINHOUSE⁴ in liver slices of rats made hyperthyroid. These authors found that the rate of oxidation of acetate was 30–70% higher in liver slices of thyroid hormone-treated rats. They also reported that contrary to expectation acetate incorporation into fatty acids in these treated rats was as high as in normal animals or higher.

Investigation on the synthesis of cholesterol in hyperthyroidism has been somewhat more extensive. Several authors have concluded that cholesterol synthesis is stimulated in hyperthyroid states while it appears depressed in the opposite condition^{5–7}.

¹ Supported in part by grants-in-aid from the John A. Hartford Memorial Fund; the National Institute of Arthritis and Metabolism (Gr. No. A-49) Public Health Service, Bethesda; the Albert and Mary Lasker Foundation, New York; the Nutrition Foundation, New York; and the Fund for Research and Teaching, Dept. of Nutrition, Harvard.

² Holder of a Research Fellowship in Department of Nutrition, Harvard School of Public Health, July 1957 to December 1958.

³ Department of Nutrition, Harvard School of Public Health, Boston (Mass.).

⁴ M. A. SPIRITES, G. MEDES, and S. WEINHOUSE, *J. biol. Chem.* **204**, 705 (1953).

⁵ R. H. ROSENMAN, S. O. BEYERS, and M. FRIEDMAN, *J. clin. Endocrin.* **12**, 1287 (1952).

⁶ W. MARX, S. T. GUSTIN, and C. LEVI, *Proc. Soc. exp. Biol. Med.*, **N. Y.** **83**, 143 (1953).

⁷ M. A. SPIRITES, G. MEDES, and S. WEINHOUSE, *XIXth Intern. Physiol. Congr. Abstr. Montreal (1953)*, p. 789.

It has been customary to study the effect of thyroid hormones in synthetic processes by including thyroid preparations in the diet. However, single determinations after chronic treatment are difficult to interpret as secondary physiological reactions may interfere with the initial effect of thyroxine. It seemed useful to determine the course of fatty acid and cholesterol synthesis from acetate after subcutaneous administration of a single dose of Thyroxine. A study of the effect of prolonged treatment is also included.

Materials and Methods. The animals used were adult male albino mice. They were maintained in individual cages and fed *ad libitum* Purina chow and water. All animals were kept fasting for 24 h before being killed.

Each group consisted of experimental animals and controls. Experimental animals received a subcutaneous injection of 100 μ g of l-Thyroxine. Controls received an equal volume of saline by the same route. At the appropriate time all animals were injected intraperitoneally with 0.4 mg of Na acetate-C¹⁴ (an approximate total of 10⁶ counts/min to each animal). 30 min later they were killed by a blow on the head and decapitation. The liver was excised and weighed separately from the carcass.

One group of animals received 1.8 mg of l-Thyroxine at 24 h intervals over a period of 28 days. An equal number of controls were injected with saline in the same way.

Thyroxine solution was prepared by dispersing the powder (1-sodium thyroxine pentahydrate, Smith, Kline, and French) in small amounts of 0.9% saline and adding 1 N sodium hydroxide until complete solubility was achieved. The pH was then adjusted to 8 with 1 N HCl and 0.9% saline added to obtain the desired volume. All injections were administered subcutaneously in volume of 0.2 ml.

Extraction and Determination of Fatty Acids and Cholesterol. The method followed was essentially that described in a previous publication⁸. Results are expressed as percentage of counts retained $\times 10^3$.

⁸ C. E. ZOMZELY and J. MAYER, *Amer. J. Physiol.* **187**, 365 (1956).

Table I. Effect of Thyroxine on Hepatic Lipogenesis and Cholesterologenesis from Labelled Acetate

Interval Tx-sacrification	Groups (Number of animals)	Liver				
		Weight (g)	g Fatty acids per 100 g liver	% retention in liver Fatty acids $\times 1000$	mg Cholesterol per g of liver	% retention in liver Cholesterol
6 h	Controls (7)	0.98 $\pm$ 0.06	9.41 $\pm$ 2.1	13.4 $\pm$ 3.6		
	Treated (7)	1.02 $\pm$ 0.12	8.94 $\pm$ 2.9	17.7 $\pm$ 6.8		
16 h	Controls (10)	1.19 $\pm$ 0.14	5.22 $\pm$ 1.7	20.2 $\pm$ 5.0	1.87 $\pm$ 0.51	9.1 $\pm$ 7.6
	Treated (10)	1.38 $\pm$ 0.25	5.25 $\pm$ 2.4	31.4 $\pm$ 19*	2.37 $\pm$ 0.35	20.8 $\pm$ 19
24 h	Controls (10)	1.55 $\pm$ 0.15	6.79 $\pm$ 1.9	24.7 $\pm$ 3.8	2.67 $\pm$ 0.41	32.0 $\pm$ 18
	Treated (10)	1.66 $\pm$ 0.18	8.80 $\pm$ 4.8	41.3 $\pm$ 8.6++	2.09 $\pm$ 0.20	161 $\pm$ 155**
32 h	Controls (10)	1.42 $\pm$ 0.23	6.83 $\pm$ 2.5	19.1 $\pm$ 8.0	2.57 $\pm$ 0.32	24.2 $\pm$ 16
	Treated (10)	1.48 $\pm$ 0.30	10.64 $\pm$ 4.2	23.6 $\pm$ 12	2.18 $\pm$ 0.56	104 $\pm$ 85+
48 h	Controls (10)	1.68 $\pm$ 0.47	8.69 $\pm$ 4.3	20.6 $\pm$ 6.6	2.45 $\pm$ 0.53	36.1 $\pm$ 19
	Treated (10)	1.64 $\pm$ 0.33	9.40 $\pm$ 5.5	22.0 $\pm$ 14	1.91 $\pm$ 0.51	141 $\pm$ 122***
Chronic Expt.	Controls (10)	1.48 $\pm$ 0.31	4.35 $\pm$ 2.0	13.3 $\pm$ 3.9	2.57 $\pm$ 0.40	31.0 $\pm$ 16
	Treated (9)	2.02 $\pm$ 0.40+	3.43 $\pm$ 1.9	59.9 $\pm$ 32++	1.88 $\pm$ 0.49	39.1 $\pm$ 51

* $p < 0.10$ ** $p < 0.05$ *** $p < 0.02$ + $p < 0.01$ ++ $p < 0.001$

Results expressed as means S.D. in the livers of 6 groups of fasting mice, each consisting of treated animals and controls. The treated mice of the first 5 groups received 100 mg of Thyroxine subcutaneously and were sacrificed with the controls (injected with saline in the same manner) at various intervals 30 min after an intraperitoneal injection of acetate- C^{14} . The treated mice of the last group received 1.8 mg of L-Thyroxine over a period of 28 days.

Table II. Effect of Thyroxine on Carcass Lipogenesis and Cholesterologenesis from Labelled Acetate

Interval Tx-sacrification	Groups (Number of animals)	Carcass				
		Weight (g)	mg Fatty Acids per 100 g carcass	% retention in carcass Fatty acids $\times 1000$	mg Cholesterol per 100 g carcass	% retention in carcass Cholesterol $\times 1000$
6 h	Controls (7)	21.1 $\pm$ 1.5	10.8 $\pm$ 3.9	260 $\pm$ 66		
	Treated (7)	21.1 $\pm$ 1.4	10.8 $\pm$ 3.7	362 $\pm$ 84**		
16 h	Controls (10)	21.9 $\pm$ 1.9	7.5 $\pm$ 3.9	268 $\pm$ 32	241	290 $\pm$ 48
	Treated (10)	21.3 $\pm$ 2.1	6.1 $\pm$ 1.7	341 $\pm$ 57++	230	328 $\pm$ 56
24 h	Controls (10)	24.1 $\pm$ 1.9	6.5 $\pm$ 1.7	296 $\pm$ 53	226	321 $\pm$ 72
	Treated (9)	24.8 $\pm$ 1.6	7.1 $\pm$ 2.2	287 $\pm$ 52	244	403 $\pm$ 90**
36 h	Controls (10)	24.7 $\pm$ 2.3	7.9 $\pm$ 3.0	257 $\pm$ 62	257	298 $\pm$ 58
	Treated (9)	24.1 $\pm$ 2.0	7.8 $\pm$ 3.0	262 $\pm$ 50	256	267 $\pm$ 28
48 h	Controls (10)	25.4 $\pm$ 1.7	8.2 $\pm$ 3.9	227 $\pm$ 40	244	301 $\pm$ 67
	Treated (10)	25.5 $\pm$ 2.2	9.5 $\pm$ 5.4	221 $\pm$ 19	238	298 $\pm$ 41
Chronic Expt.	Controls (10)	25.6 $\pm$ 2.6	5.3 $\pm$ 2.1	236 $\pm$ 32	278	378 $\pm$ 96
	Treated (9)	28.2 $\pm$ 2.2	4.0 $\pm$ 2.3	209 $\pm$ 34	266	280 $\pm$ 30+

* $p < 0.10$ ** $p < 0.05$ *** $p < 0.02$ + $p < 0.01$ ++ $p < 0.001$

Results expressed as means S.D. found in the carcasses of the same animals as in Table I

Results. Hepatic incorporation of acetate-C¹⁴ into fatty acids and cholesterol after one subcutaneous dose of 100 g of l-Thyroxine. As can be seen from Table I hepatic lipogenesis appears susceptible of being stimulated by one dose of Thyroxine. 24 h after administration of a single dose the rise in lipogenesis was 70% and highly significant. Within 48 h the effect had practically disappeared as can be readily observed from Table I. The % retention of acetate-C¹⁴ into liver cholesterol appears to be also increased by one subcutaneous injection of Thyroxine. A 3-5-fold increase was observed between 24 and 48 h after the hormone administration. Contrary to what was observed for fatty acid synthesis, the effect of thyroxine on liver cholesterogenesis appears to be more persistent: the increase in rate of cholesterogenesis was still several times the normal rate after 48 h. In spite of the fact that the significance of the increase is partly obscured by the wide range of values, it seems possible to conclude that at 24, 36, and 48 h after Thyroxine administration liver cholesterogenesis is decidedly higher than in the control group.

Carcass incorporation of acetate-C¹⁴ into fatty acids and cholesterol after one subcutaneous dose of 100 g of l-Thyroxine. Table II shows carcass retention of acetate-C¹⁴ into fatty acids to be affected in the way of an increase 6 and 16 h after Thyroxine injection, while the incorporation into carcass cholesterol, except for a small and transient rise found 24 h after the administration of the hormone was not affected in the treated groups at other time intervals.

Hepatic incorporation of acetate-C¹⁴ into fatty acids and cholesterol after repeated administration of l-Thyroxine. The animals which received 1.8 mg of l-Thyroxine over a period of 28 days did not show some of the 'classic' signs of hyperthyroidism. Their weight was increased significantly ($p < 0.01$) over that of the controls and their fur appeared more luxuriant and cleaner than that of the controls. Food intake was increased. While the increase in body weight was in part due to the increase in liver weight, the weight of the carcass was also greater at the end of treatment. Hepatic lipogenesis was increased four-fold. The increase was highly significant as is readily seen from Table I. While, as mentioned above, liver weight was increased, the fatty acid content per 100 g of liver was significantly lower indicating a faster turnover of hepatic fatty acids. Table I also shows that contrary to what could be expected, cholesterol synthesis *in vivo* following chronic administration of Thyroxine was not increased.

Carcass incorporation of acetate-C¹⁴ into fatty acids and cholesterol after repeated administration of l-Thyroxine. Table II shows that following prolonged thyroxine administration carcass lipogenesis was not affected, while cholesterol synthesis tended to be depressed.

Discussion. In all experiments the animals were kept fasted for 24 h. The duration of fasting had been chosen so as to minimize the extremely wide range of individual variability seen when lipogenesis and cholesterogenesis are measured in the fed state. Under these conditions, administration of a single dose of thyroxine appeared to promote within 24 h a 79% increase in hepatic synthesis of fatty acids and a 3-5-fold increase in the hepatic synthesis of cholesterol.

A major limitation in interpreting these experiments is that data on retention of acetate-C¹⁴ into fatty acids and

cholesterol does not provide a satisfactory measure of lipogenesis and cholesterogenesis unless the specific activity of the pool of acetyl groups is known at the site of synthesis. It is possible that the apparent rates of synthesis are in part function of the variations in the dilution of the administered acetate-C¹⁴ by endogenously produced acetate. Such a possibility has been examined experimentally in studies of lipogenesis and cholesterogenesis in the hereditary obese hyperglycemic syndrome and in hypothalamic obesity in mice⁹. In a situation such as the one created by thyroid treatment where increased oxygen consumption represents the most evident and rapid feature even after the administration of one single dose, a reduced acetyl pool is hardly to be expected. It is likely that the results would be even more significant because of increased size of acetate pool in the treated groups if the size of these pools had in fact been determined.

It seems therefore reasonable to conclude that one effect of thyroxine is that it increases the rate of fatty acid synthesis in the mouse liver. This in turn may be coupled with or rather be the result of an increased glucose oxidation as both liver glucose uptake by isolated rat liver¹⁰ and glucose oxidation in rat liver slices¹¹ have been found to be increased by a period of thyroid treatment. GLOCK *et al.*^{11,12} have also found significantly higher levels of activity of glucose-6-phosphate and 6-phosphogluconate dehydrogenases in the livers of thyroid treated rats. This would support the possibility that the increased lipogenesis might find its explanation in an increased HMP shunt activity. However, further investigations where utilization of 1-¹⁴C-glucose and 6-¹⁴C-glucose liver slices were determined by measuring the conversion of these labelled substrates into ¹⁴CO₂ failed to show comparatively greater participation of the HMP shunt in the liver slices of the thyroid treated rats. Total liver TPN and principally TPNH were strikingly reduced as a result of thyroxine treatment. Thus, while the increase in lipogenesis in the thyroxine-treated animals is in agreement with the general concept that lipogenesis is coupled with an increased glucose utilization, the mechanism of the stimulation remains obscure. Explanation of the fact that after initially stimulating cholesterogenesis, continued thyroxine treatment brings cholesterogenesis back to pre-treatment levels also requires further experimental work.

Résumé

L'effet d'une dose sous-cutanée de l-Thyroxine sur des souris à jeun consiste en une augmentation progressive de la lipogénèse et de la cholestérogénèse hépatiques. Cette augmentation atteint 70% pour la lipogénèse après 24 h et 300-500% pour la cholestérogénèse après 24, 36 et 48 h.

Un traitement chronique (28 j) cause une augmentation (400%) de la lipogénèse, mais non de la cholestérogénèse hépatiques.

Les effets de la thyroxine sur la lipogénèse et la cholestérogénèse extra-hépatiques sont beaucoup moins nets.

⁹ C. E. ZOMZELY and J. MAYER, *Amer. J. Physiol.* **196**, 956 (1959).

¹⁰ S. D. BURTON, E. D. ROBBINS, and S. O. BEYERS, *Proc. Soc. exp. Biol. Med.*, N.Y. **92**, 272 (1956).

¹¹ G. E. GLOCK, P. McLEAN, and J. K. WHITEHEAD, *Biochem. J.* **63**, 520 (1956).

¹² G. E. GLOCK and P. McLEAN, *Biochem. J.* **61**, 390 (1955).

EXPERIENTIA MAIORUM

The Discovery of Atmospheric Condensation Nuclei by Paul-Jean Coulier in 1875

A historical note

By F. VERZAR*

Nobody will doubt the great value of JOHN AITKEN'S¹⁻⁴ work of several decades on atmospheric condensation nuclei as the cause of haze, fog, and clouds, but all the basic observations were made without AITKEN's knowledge several years before by PAUL-JEAN COULIER, professeur au Val de Grâce, the military medical and pharmaceutical school of Paris.

In the *Journal de Pharmacie et de Chimie* (Ser. IV, 22, 165, September 1875), appeared a paper entitled *Note sur une nouvelle propriété de l'air* par M. COULIER⁵, professeur au Val de Grâce.

The Figure shows the first page.

COULIER describes a simple device for demonstrating the fact, which was already known at the time, that a sudden decrease in pressure in a closed volume of air, saturated with water vapour, leads to the formation of fog. Originally he used a 3-meter-long tube of zinc closed at both ends with a glass plate. The air inside was compressed first. It was then sufficient to open a stop-cock to decrease the pressure, and at this moment a fog appeared which was so thick that a candle flame could no longer be seen through the tube. (This certainly sounds like the model of our modern nucleus counters, but – of course – without photocells and electronics.)

A second device was also described. A flask with 3 stop-cocks was used. One was connected with a rubber balloon. The air was sucked in and, by compression of the balloon, was brought under higher pressure which could then be released at will, and then a fog appeared.

He even observed the fog droplets with a simple magnifying glass and saw that they are of different size and mobility. He also started counting the nuclei number per cm³. He found that the air of Paris contains many such dust particles – as he called them – which he supposed to be responsible for the 'bad air' of the big city.

He then makes the interesting observation that after several decompressions of the same volume of air, it becomes 'inactive'. Oxygen and carbon dioxide do not play a part in this. With outside air, the reaction (fog formation) appears again.

Through a cotton-wool filter, the particles of the air can be filtered away and such air then becomes 'inactive', that is no fog is produced by sudden decrease in pressure.

These invisible particles sediment when the air stands. They are also retained in the respiratory tract (bronchi). Tobacco smoke makes air 'extraordinarily active'. An alcohol flame or a benzol flame, or a Bunsen burner, produces very much 'activity', and these particles are retained by cotton wool filters. Since the latter become

black, he concludes that the dust-producing particles are very small, unburned particles of carbon. A calculation showed him that as little as $7/100\,000$ mg 'activate' the air.

Note sur une nouvelle propriété de l'air; par M. COULIER, professeur au Val-de-Grâce.

On sait que lorsqu'une certaine quantité d'air saturé de vapeur d'eau est raréfiée brusquement, une partie de cette vapeur se précipite sous forme de brouillard, par suite de l'abaissement de température. — Pour rendre cette expérience plus visible, j'ai fait construire un large tube en zinc, de 3 mètres environ de longueur, et terminé par des glaces. En introduisant un peu d'eau dans cet appareil et en comprimant légèrement l'air qu'il contient, il suffit d'ouvrir un robinet placé sur le côté du tube pour que la décompression se produise. A ce moment un nuage se forme, et il est assez opaque pour qu'on ne puisse plus voir les contours de la flamme d'une bougie.

En répétant cette expérience à plusieurs jours d'intervalle, je m'aperçus qu'elle était capricieuse, et que souvent elle ne réussissait pas. C'est pour rechercher les causes de cet insuccès que j'ai entrepris les expériences dont je vais dire quelques mots.

Le phénomène de la précipitation de la vapeur d'eau dans une atmosphère qu'on raréfie présente une particularité qui permet de l'étudier plus facilement. On peut, pour le produire, provoquer dans l'air saturé une compression momentanée qui ne dure qu'un instant très-court. Le brouillard se produit immédiatement au moment de la décompression. L'appareil suivant est fort commode pour ce genre d'expérience. Il se compose d'un flacon de 1 à 2 litres, à trois tubulures (A, fig. 1). La première, B, est munie d'un robinet et se termine par un tube en caoutchouc. La deuxième, C, est dépourvue de robinet,

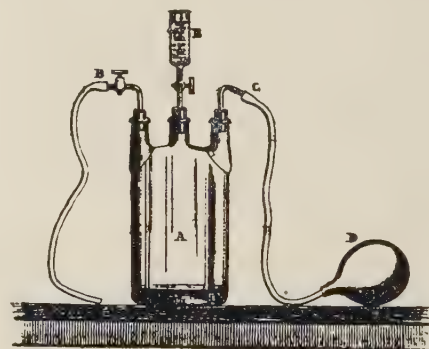


Fig. 1.

mais le tube en caoutchouc se termine par une poire, D.

He further showed that the number of nuclei changes with the weather, and he therefore proposed that in towns, and also at different altitudes, and during epidemics (!), the presence of these 'dust' particles should be measured and their quality studied.

Ozone produced great activity in his air samples, and this active principle was not retained by the cotton wool filters. This fact remained inexplicable and is today again under discussion (JUNGE⁶).

* Basel.

¹ J. AITKEN, *Trans. R. Soc. Edinburgh*, Dec. 20th (1880).² J. AITKEN, *Nature* 23, 195 (1881).³ J. AITKEN, *Nature* 23, 384 (1881).⁴ J. AITKEN, *Collection of Scientific Papers* (University Press, Cambridge 1923).⁵ M. COULIER, *J. Pharm. Chim.* [4] 22, 165, 254 (1875).⁶ C. E. JUNGE, *Adv. Geophys.* 4, 1 (1958).

Of special interest is his remark that 'Sulphur was the most active fog-producing substance'. In recent years, GERHARD and JOHNSTONE⁷ and JUNGE⁶ have discussed the role of SO₂ in the atmosphere and its possible oxydation to sulphuric acid by ozone and by a photochemical reaction (VERZÁR^{8,9}).

In the October number of the same journal where COULIER's⁵ first paper appeared, followed a second additional note with a similar title, of 1½ pages only. In this he was much perturbed whether his original interpretation, that the dust nuclei are mainly combustion products, was right. He now had made the following observations: The flask was filled with inactive (filtered) air. In the bottle was a platinum wire, which was brought to a red glow. The air then became very active. This and some more experiments with heated glass-tubes etc. made it uncertain whether his explanation was right that carbon particles are the nuclei on which water vapours condense. Here a new form of nuclei were produced which – he thought – were not products of burning organic matter.

It cannot be said that COULIER's papers were quite unknown; the German scientific weekly periodical 'Naturforscher' gave a lengthy review in Vol. 8 (1875), p. 400 to 401, and a second one on p. 453.

However, 5 years later, AITKEN¹ published in the Royal Society of Edinburgh on December 20th, 1880, his famous paper in which he showed that 'the dust is the germ on which fogs and clouds are the developed phenomena', as it was quoted in Nature 23, December 30th (1880), p. 195 and p. 204.

This led to a series of critical 'Letters to the Editor' in Nature, by RUSSELL¹⁰, PREECE¹¹, and mainly one by GRONEMAN¹² in Groningen (Holland). The latter pointed out the importance of COULIER's work on p. 337 as primary to that of AITKEN, but GRONEMAN was interested in the problem only from the point of view of his 'théorie cosmique de l'aurore polaire'.

AITKEN³ answered in a letter to the Editor and fully acknowledged that he had not known of COULIER's work: 'I need not say that the information... was a most unexpected surprise. Nothing whatever seems to have been known in England about the results obtained ... There be no doubt that M. COULIER was the first to show the important part played by dust in the cloudy condensation of the vapour in air.' AITKEN thought, however, that COULIER's second paper, in which he showed that a heated glowing platinum wire also produced condensation nuclei, was the cause that no further interest had been taken in his work. AITKEN himself explained this experiment so that some dirt must have been present which burned on the wire, and thus no special explanation of the origin of the nuclei would be necessary.

Today we see the situation differently: Only recently O'CONNOR *et al.*¹³ have studied the nuclei produced from glowing metal wires and found that they have a radius of 1×10^{-6} cm; but their nature is still unknown today. Thus COULIER made an important new observation even in this second short note of 1875.

It seems astonishing that COULIER did not continue this most promising completely new work. The explanation is that less than one year after his most important discoveries, in 1876, he left his chair of chemistry and accepted an administrative position as 'Inspecteur' in the army medical services¹⁴. Obviously he even did not take notice of AITKEN's work on his problems during the next 15 years.

PAUL-JEAN COULIER was born in Paris on August 31st, 1824. He was a pharmacist and became in 1853 professeur agrégé on the 'Ecole de médecine et de pharmacie militaire du Val de Grâce' in Paris and from 1858 professor of applied chemistry and hygiene.

From his 30 papers the earlier dealt with blood and viruses, the later with hygiene of soldiers, heating and ventilation¹⁵. This obviously lead him to study formation of fogs. After 1876, when he took up his new post, he was especially known for his fights for the liberty of medical opinion in military service. He died greatly honoured, on July 23rd, 1890.

Few know his name today, but he is mentioned in later reviews. (LANDSBERG¹⁶, p. 160 and p. 172, MASON¹⁷, GREEN, and LANE¹⁸.)

COULIER should be remembered as the first who showed with ingeniously simple methods, and few words, the existence, the rôle and most of the basic facts of atmospheric condensation nuclei.

I have to thank Médecine-Colonel HASSENFORDER of the Musée du Val de Grâce, and the Bibliothèque centrale du service de santé, for the biographical data.

Zusammenfassung

1875 hat PAUL-JEAN COULIER, Professor für Chemie und Hygiene an der Medizinischen Militärschule Val de Grâce in Paris, mit einfachen Mitteln die Rolle der atmosphärischen Kondensationskerne bei der Nebelbildung entdeckt und viele wesentliche Tatsachen beschrieben. Erst 5 Jahre später erfolgte die erste Arbeit von AITKEN. Die Geschichte dieser Entdeckung und die Ursache dafür, dass sie nicht besser bekannt wurde, wird besprochen.

¹⁴ CHARLES LAVANZALLE, *Notices Biographiques sur les anciens inspecteurs de l'armée* (Paris 1892), p. 35.

¹⁵ A. BALLAND, *Travaux scientifiques des pharmaciens militaires français* (Edit. Asselin, Paris 1882), p. 26.

¹⁶ H. E. LANDSBERG, *Erg. Kosm. Phys.* 3, 155 (1938).

¹⁷ B. J. MASON, *The Physics of Clouds* (Oxford 1957), p. 20.

¹⁸ H. L. GREEN and W. R. LANE, *Particulate Clouds, Dusts, Smokes and Mists* (London 1957), p. 231.

COGITATIONES

Rauschzustände nach Pilzgenuss

Bei der Eroberung von Mexiko lernten die Spanier in den südlichen Provinzen eigenartige Kulthandlungen kennen, während derer sich die Teilnehmer durch den Genuss von Hutzpilzen in Trance versetzten. Pater DE OLMOS berichtet schon um das Jahr 1543, dass junge indianische Männer und Töchter zur Erbauung der Götter in blumengeschmückten Tempeln tanzten, wobei der Satan sie einen berausenden Pilz einnehmen liess, so dass sie ihre Sinne verloren und Visionen sahen.

⁷ E. R. GERHARD and H. F. JOHNSTONE, *Ind. Eng. Chem.* 47, 972 (1955).

⁸ F. VERZÁR and Y. KUNZ, *Geofis. pur. appl.* 38, 215 (1957).

⁹ F. VERZÁR, *Geofis. pur. appl.*, in press (1959).

¹⁰ R. RUSSELL, *Nature* 23, 267 (1881).

¹¹ W. H. PREECE, *Nature* 23, 194 (1881).

¹² M. H. J. H. GRONEMAN, *Nature* 23, 337 (1881).

¹³ T. C. O'CONNOR, W. P. F. SHARKEY, and C. O'BROILCHAIN, *Geofis. pur. appl.* 42, 109 (1959).

Den Indianern jener Zeit waren diese Pilze heilig; denn sie öffneten ihnen die Pforte zu einer überirdischen Welt der Gesichte und Wunder, ein Ausgleich zum grauen Alltag. Entsprechende Darstellungen in Skulpturen, Reliefs und Wandmalereien lassen sich bis in das 5. Jahrhundert v. Chr. zurückverfolgen. Seit der Zertrümmerung der Mayakultur wurden sie aber – bis zum Erscheinen des hier anzuzeigenden Buches – nicht mehr verstanden. Ethnographische Belege von «heiligen Pilzen» fanden ihren Weg auch nach Europa; so ist in der Sammlung von der Heydt im Rietbergmuseum in Zürich eine Steinplastik aus dem 4.-6. nachchristlichen Jahrhundert ausgestellt: ein gigantischer Hutpilz, aus dessen Stiel der Kopf eines Gottes, umgeben von einer neunzackigen Aureole, hervorbricht; die Jäger träufelten jeweils das Blut junger Vögel oder junger Hirsche in den Mund des Steines; sobald es von den Göttern getrunken war und die Priester und Kultbeamten sich mit ihren Opfergaben näherten, fing der Stein zu sprechen an.

Durch die Christianisierung des Landes wurden die heidnischen Kulte und mit ihnen das geheime Wissen um die beglückenden Drogen in entlegene Gebirgsgegenden zurückgedrängt; doch liess sich die Kunde, dass es bis nach Zentralamerika hinein zauberkräftige «heilige Pilze» gebe, nicht auslöschen. Der Wirklichkeitsgehalt dieser vagen Berichte wurde in den vergangenen Jahren unter der Leitung von G. WASSON (New York) auf ausgedehnten ethnographischen Reisen überprüft und hernach unter der Leitung von ROGER HEIM (Paris) naturwissenschaftlich bearbeitet. Es gelang den Forschern, in einsamen Siedlungen das Vertrauen der indianischen Urbevölkerung und insbesondere der «Wissenden», das heisst der indianischen Zauberer, zu gewinnen. Rauscherzeugende Pilze sind den Einheimischen auch heute noch bekannt und werden von ihnen, 400 Jahre nach ihrer Bekehrung zum Christentum, noch immer bei geheimen kultischen Handlungen verwendet; daneben dienen sie auch dem persönlichen Gebrauch zu therapeutischen Zwecken oder als Narkotika. Die beiden Autoren konnten sogar einigen nächtlichen Feiern beiwohnen und sie photographieren; sie legen ihre Ergebnisse in einem gewichtigen Quartband vor¹.

Das Wissen, dass bestimmte Hutpilze rauschähnliche Zustände auszulösen vermögen, ist über die ganze Erde verbreitet; so wird in Sibirien eine besondere Rasse unseres Fliegenpilzes in getrocknetem Zustande genossen, um zu einer dem Alkoholrausch ähnlichen psychischen Steigerung zu gelangen.

Neu ist jedoch die Mannigfaltigkeit der halluzinogenen Pilze in Mexiko. ROGER HEIM beschreibt nicht weniger als

13 Arten, von denen die Mehrzahl für die Wissenschaft neu sind; sie gehören zum Teil den Gattungen *Stropharia* und *Psilocybe* an, die in andern Arten auch bei uns vorkommen. Eine der mexikanischen Arten wurde sogar für Cochinchina nachgewiesen.

Neu ist ferner, dass diese halluzinogenen Pilze schon vor Jahrtausenden fest in religiöse Riten eingefügt und in dieser Eigenschaft in kultischen Gegenständen, in figuralen Darstellungen usw. verewigt wurden; sie müssen somit im Rahmen dieser Riten eine zentrale Bedeutung besessen haben.

Und neu ist endlich der Wirkstoff, der von einer Forschergruppe der Sandoz AG. in Basel (A. CERLETTI, A. HOFMANN, J. RENZ und ihren Mitarbeitern) isoliert, in seiner chemischen Konstitution aufgeklärt, hernach synthetisiert und in pharmakologischer Hinsicht abgeklärt wurde; es handelt sich um das *Psilocybin*, das Orthophosphat einer neuartigen Indolverbindung, einen entfernten Verwandten der Lysergsäure und der Mutterkornalkaloide.

Die psychophysiologischen und klinischen Wirkungen dieses Stoffes wurden an der Faculté de Médecine in Paris unter der Leitung von J. DELAY bei einigen Dutzend gesunden und geisteskranken Individuen untersucht und zusätzlich von den beteiligten Forschern im Selbstversuch überprüft. Sie sind breit und umfassen im psychischen Sektor alle möglichen Steigerungen, so (je nach dem Individuum) eine gewisse Euphorie oder eine Geschwätzigkeit, sinnloses Lachen, Halluzinationen, farbige Visionen usw.; im somatischen Sektor erfolgt dagegen eine Herabsetzung der Pulszahl, des Blutdruckes und allgemein des Tonus. Doch scheint es dem Referenten als Nichtfachmann, dass die Indianer Mexikos und Zentralamerikas auf diese halluzinogenen Stoffe stärker angesprochen haben müssen, als wir Europäer es vermögen; wenn nämlich der Rausch bei ihnen nicht grösser und beglückender gewesen wäre als bei unsern Pariser und Basler Kollegen im Selbstversuch, so wären diese Drogen nicht in den Mittelpunkt ganzer Kulthandlungen gerückt worden. Es ist offenbar ein Rassenmerkmal von uns Europäern, dass wir uns auch im Trancezustand nicht leicht entpersönlichen können.

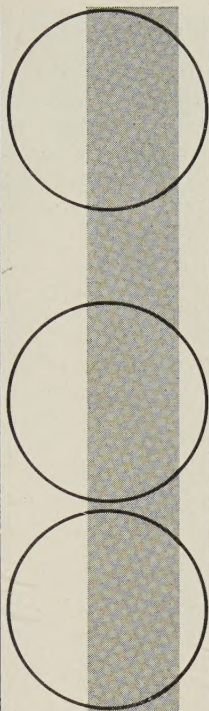
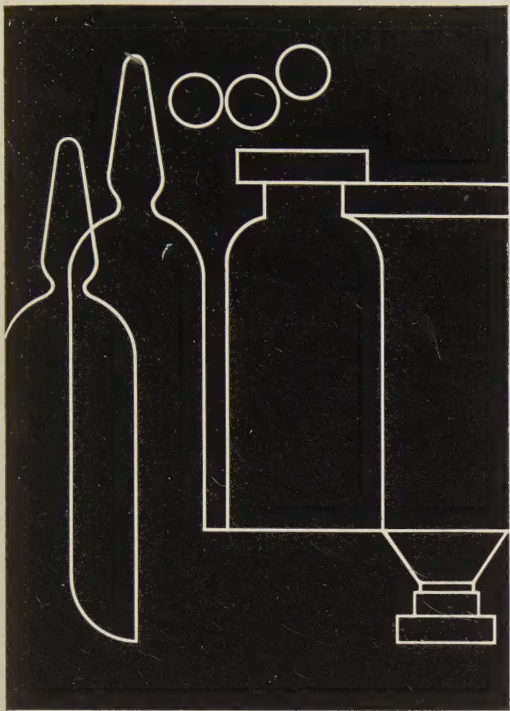
E. GÄUMANN

Institut für spezielle Botanik, ETH, Zürich, 2. Juni 1959.

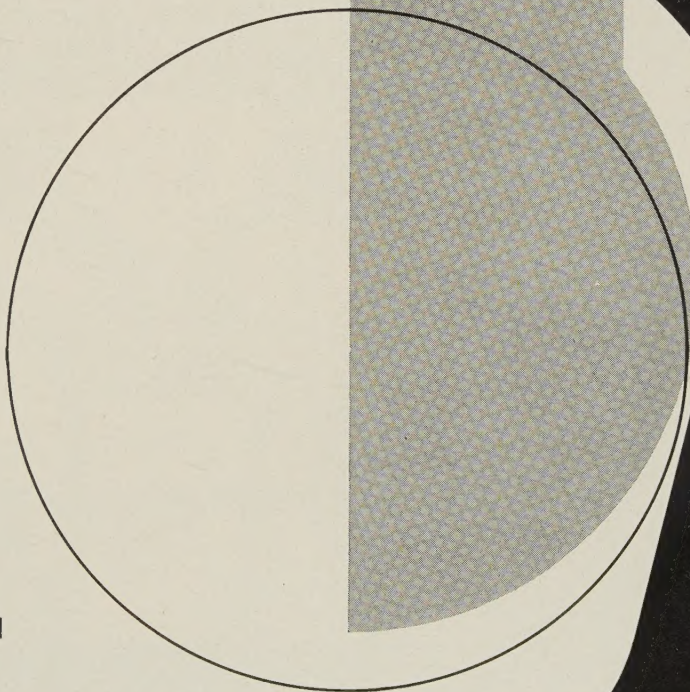
Summary

In their work, R. HEIM and R. G. WASSON have reported on the 'Myco-Ethnology' of hallucinogenic mushrooms in Mexico. Further attention was paid to the taxonomy of different species of these mushrooms belonging to the genera *Psilocybe*, *Stropharia* etc. Data on the elucidation and synthesis of the active principle, *Psilocybin*, a new indole derivate, are given.

¹ R. HEIM et R. G. WASSON, *Les champignons hallucinogènes du Mexique*. Archives du Muséum d'Hist. nat. de Paris, 7^e sér., 6, 1958, erschienen 1959, 324 S., 86 farbige bzw. schwarz-weiße Abbildungen und Karten im Text, 37 farbige bzw. schwarz-weiße Tafeln, Preis 64 Dollar.



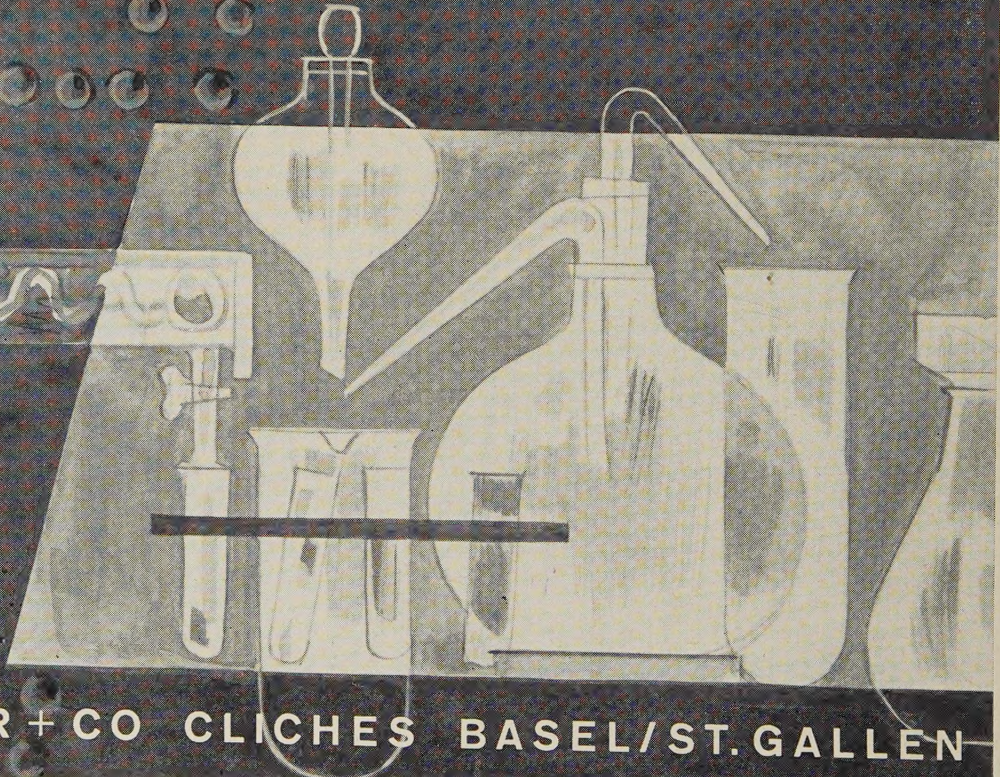
**Die pharmazeutischen
Spezialitäten SANDOZ
geniessen
das Vertrauen
der Ärzte
in der ganzen Welt**



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